THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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AUGUST TO DECEMBER, 1933

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EDITOR'S NOTE:

Paragraph 4 of the summary of the article by Nellie M. Payne on p. 204 should read as follows:

"In general high temperatures (above 32° C.) favor the parasite, low temperatures (15° C. and below) the host."

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FORTY-FIFTH YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the

President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is submitted my report as Treasurer of the Marine Biological Laboratory for the year 1932.

The accounts have been audited by Messrs. Seamans, Stetson, and Tuttle, certified public accountants, and a copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1932, the book value (cost) of the general Endowment Fund in the hands of the Central Hanover Bank and Trust Company as Trustee, was $906,415 in securities, and $311.49 in cash.

The book value (cost) of the Library Fund, was $195,949.19 in securities, and $127.56 in cash.

All of the income due during the year on the general Endowment Fund and on the Library Fund was collected with the exception of interest amounting to $791.00 on a mortgage belonging to the general Endowment Fund.

The Reserve Fund consisted of securities of the book value (cost) of $19,671.13 and cash of $1,623.59.

The Retirement Fund consisted of mortgages of the book value of $19,300 and cash of $2,413.62.

There has been no change in the investments of the other minor funds.

The land, buildings, equipment and library, excluding the Gansett and Devil's Lane tracts, represented an investment of $1,683,017.56, less depreciation of $327,455.68, or a net amount of $1,355,561.88.

Current expenses including $41,051.48 depreciation exceeded income by $24,585.55. Over $40,000 was expended from current funds in the acquisition of real property, alterations and additions to the electric system of the pumping station and Laboratory, on new equipment and on books, of which over $18,000 was expended for books.

At the end of the year the Laboratory owed $27,000 on bond and mortgage, and in accounts payable $8,921.41, most of which was a deferred liability to be paid one half in 1933 and the balance in 1934, and had $14,316.33 current funds in its bank accounts. Following is the balance sheet as of December 31, 1932, and the condensed statement of income and outgo for the year, also the surplus account.

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1932

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank & Trust Company (of New York), Trustee—Schedules I-a and I-b		$1,102,803.24	
Securities and Cash—Minor Funds—Schedule II		10,698.88	$1,113,502.12
Plant Assets:			
Land—Schedule IV	$ 98,103.05		
Buildings—Schedule IV	1,224,618.42		
Equipment—Schedule IV	167,229.03		
Library—Schedule IV	193,067.06	$1,683,017.56	
Less Reserves for Depreciation		327,455.68	
		$1,355,561.88	
Securities and Cash in Reserve Fund		21,294.72	
Cash in Dormitory Building Fund		818.96	$1,377,675.56
Current Assets:			
Cash		$ 14,816.33	
Accounts—Receivable		10,449.87	
Inventories:			
Supply Department	$ 38,310.30		
Biological Bulletin	9,062.55	47,372.85	
Investments:			
Devil's Lane Property	$ 40,782.48		
Gansett Property	5,103.29		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	21,713.62	80,299.39	
Prepaid Insurance		3,540.16	
Items in Suspense (Net)		291.39	$ 156,769.99
			$2,647,947.67

Liabilities

Endowment Funds:			
General Endowment Funds—Schedule III		$1,102,803.24	
Minor Funds—Schedule III		10,698.88	$1,113,502.12
Plant Funds:			
Donations and Gifts—Schedule III		$1,029,372.61	
Other Investments in Plant from Gifts and Current Funds		346,302.95	
		$1,375,675.56	
Mortgage, Danchakoff Estate		2,000.00	$1,377,675.56
Current Liabilities and Surplus:			
Mortgage, Devil's Lane Property		$ 25,000.00	

Accounts—Payable:			
Current	$ 1,576.41		
Hixon Electric Company, Deferred	7,345.00	8,921.41	
Woods Hole Oceanographic Institution:			
Amounts received for Purchase of Books	$ 6,000.00		
Less Payments	5,457.45	542.55	
		$ 34,463.96	
Current Surplus—Exhibit C		122,306.03	$ 156.769.99
			$2,647,947.67

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1932

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 47,013.66		$ 47,013.66
Library Fund		8,655.26		8,655.26
Gifts		400.00		400.00
Instruction	8,417.64	9,930.00		1,512.36
Research	4,006.10	13,425.00		9,418.90
Evening Lectures	182.50		182.50	
Biological Bulletin and Membership Dues	8,485.33	9,929.71		1,444.38
Supply Department—Schedule V	46,644.84	43,944.55	2,700.29	
Mess—Schedule VI	24,211.48	26,821.84		2,610.36
Dormitories—Schedule VII	31,732.05	12,933.23	18,798.82	
(Interest and Depreciation charged to above Three Departments. See Schedules V, VI, and VII)	*36,026.61*			36.026.61
Dividends, General Biological Supply House, Inc.		2,032.00		2,032.00
Rents:				
Danchakoff Cottages	253.43	515.00		261.57
Microscopes		458.25		458.25
Garage, Railway, etc.		345.30		345.30
Newman Cottage	69.67	200.00		130.33
Janitor's House	34.61	375.00		340.39
Interest on Bank Balances		132.90		132.90
Sales of Duplicate Library Sets		26.38		26.38
Sundries		35.24		35.24
Maintenance of Plant:				
New Laboratory Expenses	21,850.49		21,850.49	
Chemical and Special Apparatus	10,141.22		10,141.22	

Maintenance, Buildings, and Grounds	9,073.44		9,073.44	
Library Department Expenses	8,864.80		8,864.80	
Carpenter Department Expenses	1,071.11		1,071.11	
Truck Expenses	970.53		970.53	
Sundry Expense	81.07		81.07	
Workmen's Compensation Insurance	624.01		624.01	
Pumping Station Expense	114.19		114.19	
General Expenses:				
Administration Expenses	15,137.10		15,137.10	
Endowment Fund Trustee	968.50		968.50	
Interest on Loans	100.00		100.00	
Bad Debts	321.16		321.16	
Mosquito Fund Contribution	100.00		100.00	
Naples Zoological Station	250.00		250.00	
Reserve for Depreciation	41,051.48		41,051.48	
Museum Expenses	3,028.73		3,028.73	
	$201.758.87	$177,173.32	$135,429.44	$110,843.89
Excess of Expenses over Income carried to Current Surplus—Exhibit C		24,585.55		24,585.55
	$201,758.87	$201.758.87	$135,429.44	$135,429.44

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT
YEAR ENDED DECEMBER 31, 1932

Balance, January 1, 1932		$141,346.02
Add:		
Reserve for Depreciation charged to Plant Funds		41,051.48
Original cost of 216,858 square feet of land in Gansett Tract remaining on hand January 1, 1932 set up		4,977.35
		$187,374.85
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV:		
Land—Kahler Estate	$ 1,000.00	
Buildings:		
Kahler Estate	7,266.57	
Alterations and Additions to Electric System of Pumping Station and Laboratory	8,553.00	
Other Items	1,244.71	
Equipment	4,263.55	
Library Books, etc.	18,024.49	
	$40,352.32	

Pensions and Allowances Paid	$1,040.00		
Less Income of Retirement Fund	909.05	130.95	
Excess of Expenses over Income for Year as shown in Exhibit B		24,585.55	65,068.82
Balance, December 31, 1932—Exhibit A			$122,306.03

Respectfully submitted,

LAWRASON RIGGS, JR.,

Treasurer.

V. THE REPORT OF THE LIBRARIAN

The sum of $24,000 again allowed by the Executive Committee for the year 1932 was budgeted by the Librarian in figures identical with the year before. Examination of the account books kept by the Librarian will show, however, that about $1,000 in each of the years 1931 and 1932 ($1,068.68 and $1,074.16 respectively) was not expended on the Library at the end of the year. The money was not readily available for Library purposes. The Laboratory has never charged against the Library carpentry work and expenditures for permanent equipment and has always coöperated with the Library in emergency purchases. Moreover, the number of "Biological Bulletin" subscriptions allowed the Library has increased in actual money value from $1,470.15 in 1926 to $4,233.34 in 1932, transferred to the Library in the form of current serial exchanges. When the end of the year came without outstanding bills to demand the complete sum, it seemed best to follow a procedure usual in business budgeting, and to start the new year without a surplus left over from the year before. The Librarian contributed somewhat to this surplus, also, by an economy in library personnel, allowing a position made vacant in the Fall of 1932 to remain unfilled. The vacancy will remain also through 1933.

The Woods Hole Oceanographic Institution appropriation of $1,000 for the year 1932–33 (March to March) added to the balance from their $5,000, appropriated in 1930, $1,431.99 in all, was expended as follows: Books (including old), $292.54; Current Serials, $318.64; Binding, $117.92; Back Sets, $702.89; Total, $1,431.99.

At the end of the year 1932, the Library contained 36,014 volumes and 76,275 reprints. Of the volumes, 5,601 are books and 30,413 bound back sets of serials. Of the reprints, 2,500 are bound and 73,775 in pamphlet form protected in boxes. The estimate of volumes is made direct from the accession stamp, that of the reprints from count. In the first report, that of 1919–20, containing a statement of the total holdings of the Library, the reprints, which had heretofore been entered in acces-

sion with the books and serials and in the same sequence, were segregated as pamphlets, and the accession number used only for a count of books and bound serial volumes. A round number, 5,000, was subtracted from the accession number to make the record of volumes correct. Since that time, we have not counted the reprints as among the volumes of the Library, not even in cases where they are bound, and many of them of good size, 200–500 pages. When the reprints that are bound, whether pamphlet size or book size, reach 5,000, the subtraction of 5,000 from our accessioned volumes can be cancelled and from that point on, all bound reprints be automatically counted by the accession stamp (an R1–R5,000 count is being made now in the reprints as they are being bound). It will be a great satisfaction to reach the 5,000 mark in bound reprints because from that point on the accession number will at any moment show the actual number of bound volumes in the Library. None-the-less, a separate count each year of the new books, serial binding, and reprint binding can be made automatically from our records. The record will be kept also of total reprints just as heretofore.

Twelve new serials bring the total current journals to 1,126: paid Marine Biological Laboratory subscriptions, 362; exchanges, 586; gifts, 147; paid Woods Hole Oceanographic Institution subscriptions, 31. Back sets of 86 serials were purchased: for the Marine Biological Laboratory, 48 were completed, and 31 partially filled in; for the Woods Hole Oceanographic Institution, 3 were completed, and 4 partially filled in. As we stated in the report for 1927, extensive hand-books and expeditions that continue to be issued serially, are included in their current issues in the count of current serials. Of the total number of current serials, 63 are of this nature in the Marine Biological Laboratory purchases, and 3 for the Woods Hole Oceanographic Institution. The new books added to the Library were 263; 201 (nearly all current) by the Marine Biological Laboratory and 62 by the Woods Hole Oceanographic Institution.

Dr. Metcalf's library presented by him to the Marine Biological Laboratory is the finest gift of books and reprints that the Library has ever received. During the past five years, he has sent to us 9,200 reprints, 277 books, and some very fine runs in serial back sets. This includes many publications in subjects other than Dr. Metcalf's special subjects of pelagic Tunicates and of Protozoa. Part of his card catalogue and the cases he gave with them are already in use in the reading-room and offices.

Miss Margaret Sumwalt presented 50 reprints to the Library when she left the Laboratory in the Fall. Another very acceptable gift was made through the coöperation of Dr. T. S. Palmer and Dr. Herbert

Friedmann. Ten volumes of "Auk" were presented that were needed to complete the second series of this journal. The Library is indebted to five authors who kindly presented books, and two publishers and authors in combined gift; from publishers, thirteen books were presented and we wish to acknowledge with thanks books sent by the following publishers:

P. Blakiston's Son & Company	2
Doubleday, Doran & Company, Inc.	1
Lea & Febiger	2
J. B. Lippincott Company	1
McGill University Press	1
McGraw-Hill Book Company	1
The Macmillan Company	2
Ray Long & Richard R. Smith, Inc.	2
W. B. Saunders Company	1

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-fifth session of the Marine Biological Laboratory for the year 1932.

1. *Attendance.* Reference to previous annual reports will show that the number of investigators in attendance at the Laboratory increased steadily from 176 in 1923 to a maximum of 362 in 1931—a gain of 106 per cent in 8 years. In 1932, for the first time since the World War, there was a sharp drop in the registration of investigators, which brought their number back to approximately that of 1928. This falling off in attendance is to be attributed chiefly, if not wholly, to the unfavorable financial conditions existing in most American universities which, though beginning somewhat earlier, became especially serious in 1932. It is to be noted that the decrease was proportionately much greater in the case of the younger beginning investigators and research assistants, where it amounted to approximately 20 per cent, than in that of the older so-called independent investigators in which it was only 10 per cent. This difference is what might have been expected from the fact that the enforced economies of the universities up to and including 1932 had affected most adversely the younger men. There are indications at the time of the writing of this report that university conditions in 1933 will be even worse than in 1932 and that a further decline in attendance in the class of independent investigators may therefore be anticipated. During the existence of this very real emergency it has been the policy of the Laboratory, to the extent permitted by its own resources, to reduce to a minimum the financial burdens of those of its investigators whose scientific work has already been handicapped by inadequate university support.

As contrasted with the registration of investigators, that of students in the courses in 1932 remained at its usual level, since although there was some reduction in the number of applicants, these still remained considerably in excess of the number of available places in most of the courses.

As in past years, the rise and fall of the attendance of investigators and research assistants by approximately 10-day intervals is shown in tabular form.

		1927	1928	1929	1930	1931	1932
May	30	7	15	9	6	6	8
June	10	50	64	55	50	51	54
"	20	114	140	139	153	153	127
"	30	212	240	197	208	217	172
July	10	247	281	238	253	258	225
"	20	247	282	242	250	273	245
"	30	245	272	249	253	281	248
August	10	234	250	256	254	302	257
"	20	208	226	243	245	280	236
"	30	168	183	220	204	239	190
September	10	110	112	157	122	136	129
"	20	50	43	59	44	69	58
"	30	12	14	14	8	14	13

2. *The Report of the Treasurer.* As anticipated in 1931, it has been impossible for the Laboratory to maintain its very favorable financial showing of that year. During 1932 two of its three chief sources of income have undergone marked reductions. These are first, the subscriptions received from Coöperating and Subscribing Institutions, which fell from $15,975.00 in 1931 to $11,625.00 in 1932. Knowing the serious financial problems that have had to be met by most of these institutions, the Laboratory acknowledges with especial gratitude the very generous degree of support that has been continued in spite of the present unfavorable conditions. Secondly, the loss in income from the sale by the Supply Department of biological material to schools and colleges has been of considerable magnitude. This loss is due partly to the decreased purchasing power of educational institutions and partly to the very severe competition from commercial firms, which under the present abnormal conditions are frequently willing to sacrifice fair and reasonable profits in order to be able to continue their business at all. The third chief source of revenue of the Laboratory, namely, the income received from investments, showed no great falling-off in 1932; but certain unavoidable losses from this source are anticipated in 1933. Though, on the whole, the Laboratory has fared much better during the present business depression than most other educational and scientific institutions, the need of the strictest economy has been felt, and in

the budget for 1933 approved by the Executive Committee late in the summer of 1932, and subsequently several times revised, every effort has been made to keep the expenditures of the Laboratory within its income. While the carrying out of this policy may result in some minor inconveniences to both investigators and students, it is believed that neither research nor instruction will be seriously handicapped unless conditions should become considerably worse than they are at the time of the preparation of this report.

3. *The Report of the Librarian.* The progress of the Library during the past year, especially in its relation to that of previous years, may perhaps best be shown in the tabular form adopted in previous reports.

	1925	1926	1927	1928	1929	1930	1931	1932
Serials received currently	500	628	764	874	985	1060	1080	1126
Total number of bound volumes	15000	18200	22800	26500	28300	31500	33800	36000
Reprints	25000	38000	43000	51000	59000	64000	70000	76000

Additional grateful acknowledgment is here made of the very generous gift of Dr. M. M. Metcalf mentioned above in the report of Librarian.

4. *Purchases of Property.* In March, 1932, a valuable addition to the land owned by the Laboratory was made by the purchase of the Kahler property (approximately 45 by 79 feet) facing the Eel Pond and lying between the New Dormitory and the Hubbard property acquired in 1924. The holdings of the Laboratory on East Street are now unbroken and the possibilities for the advantageous erection of new buildings in the future are greatly increased. For the present the house already on this property is being used as a men's dormitory.

5. *Changes in the Electrical System.* During the spring of 1932 important changes were made in the electrical system of the Laboratory, at a cost which, spread over a period of three years, will amount to slightly less than $15,000.00. These changes had as their chief objects: (1) increasing the capacity and flexibility of the system and in particular providing an adequate source of alternating current for the operation of the enlarged X-ray plant, etc., (2) prevention of the rapid deterioration of the storage battery, which with the growth of the Laboratory had become seriously overloaded, (3) securing a constant voltage for certain types of scientific work which were difficult or impossible to carry on with the existing arrangements, (4) provision for the operation of the salt water pumps at all hours, thereby eliminating the serious shortages of sea water which with the growth of the Laboratory were occurring with increasing frequency, (5) securing a lower cost of opera-

tion of the plant. The experience of the past summer indicates that all of these objects have been attained, and the thanks of the Laboratory are due to Dr. Pond who, with the able assistance of Mr. Larkin, so successfully handled the various complicated problems that arose in connection with this work.

6. *Technical Manager.* With the increasing complexity of the methods and apparatus used in biological work, the need has become urgent for a person who can devote a larger amount of his time to the design and care of special apparatus and to other technical needs of the Laboratory and its investigators than one in residence merely during the summer months. The Laboratory has been fortunate in securing in this capacity, with the title of Technical Manager, Dr. S. E. Pond who for 7 years has served as Custodian of Apparatus, and whose experience in this special field is almost unique. Dr. Pond will be in permanent residence in Woods Hole, where, in addition to his duties in connection with the various technical problems of the Laboratory relating to research, he will continue his own investigations in the field of calcification. This important addition to the staff of the Laboratory will do much not only to facilitate the work of its investigators during the regular summer season, but to make available its special facilities to properly qualified workers at other times of the year.

7. *Lectures and Scientific Meetings.* During the summer of 1932 there were given 10 regular evening lectures in addition to 54 shorter scientific papers in which recently completed work was reported by investigators. As in 1931, a special all-day scientific session near the end of the season, devoted primarily to work accomplished during the summer, was most successful. The Spaeth Memorial Lecture for 1932 was delivered by Professor Richard Goldschmidt of Berlin-Dahlem.

8. *Board of Trustees.* During 1932 three Trustees of long standing, Dr. H. C. Bumpus, Dr. R. A. Harper, and Dr. M. M. Metcalf, were honored by being elected Trustees Emeritus by the Corporation. The vacancies so created were filled at the same meeting by the election of the following new Trustees: Dr. W. C. Allee (Class of 1935), Dr. H. B. Bigelow (Class of 1936), and Dr. Franz Schrader (Class of 1934). It is with deep regret that record is here made of the death of Dr. William Patten of Dartmouth College, who for many years rendered valuable services to the Laboratory as a Trustee and otherwise, and who in 1930 became a Trustee Emeritus.

There are appended as parts of this report:

1. The Staff, 1932.
2. Investigators and Students, 1932.
3. A Tabular View of Attendance, 1928–1932.

4. Subscribing and Coöperating Institutions, 1932.
5. Evening Lectures, 1932.
6. Shorter Scientific Papers, 1932.
7. Members of the Corporation, August, 1932.

Respectfully submitted,

M. H. JACOBS,
Director.

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1. THE STAFF, 1932

MERKEL H. JACOBS, *Director*, Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology, the University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy, Harvard University.
E. B. WILSON, Professor of Zoölogy, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

T. H. BISSONNETTE, Professor of Biology, Trinity College.
E. C. COLE, Professor of Biology, Williams College.
B. R. COONFIELD, Instructor in Biology, Brooklyn College.
O. E. NELSEN, Instructor in Zoölogy, University of Pennsylvania.
A. W. POLLISTER, Instructor in Zoölogy, Columbia University.
L. P. SAYLES, Instructor in Biology, College of the City of New York.
A. E. SEVERINGHAUS, Assistant Professor of Anatomy, College of Physicians and Surgeons, Columbia University.

JUNIOR INSTRUCTORS

C. E. HADLEY, Assistant Professor of Biology, New Jersey State Teachers College at Montclair.
S. A. MATTHEWS, Associate in Anatomy, School of Medicine, University of Pennsylvania.

PROTOZOÖLOGY

I. Investigation

(See Zoölogy)

II. Instruction

Gary N. Calkins, Professor of Protozoölogy, Columbia University.
Rachel Bowling, Instructor in Zoölogy, Columbia University.
Robert W. Stabler, Instructor in Zoölogy, University of Pennsylvania.

EMBRYOLOGY

I. Investigation

(See Zoölogy)

II. Instruction

L. G. Barth, Instructor of Experimental Zoölogy, Columbia University.
Hubert B. Goodrich, Professor of Biology, Wesleyan University.
Benjamin H. Grave, Professor of Biology, De Pauw University.
Leigh Hoadley, Professor of Zoölogy, Harvard University.
Charles Packard, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.

PHYSIOLOGY

I. Investigation

Harold C. Bradley, Professor of Physiological Chemistry, University of Wisconsin.
Walter E. Garrey, Professor of Physiology, Vanderbilt University Medical School.
Ralph S. Lillie, Professor of General Physiology, the University of Chicago.
Albert P. Mathews, Professor of Biochemistry, University of Cincinnati.

II. Instruction

Teaching Staff

William R. Amberson, Professor of Physiology, University of Tennessee.
Philip Bard, Assistant Professor of Physiology, Harvard Medical School.
Ralph W. Gerard, Assistant Professor of Physiology, the University of Chicago.
Laurence Irving, Associate Professor of Physiology, University of Toronto.
Leonor Michaelis, Member of the Rockefeller Institute, New York City.
Margaret Sumwalt, Assistant Professor of Physiology, Woman's Medical College of Pennsylvania.

Special Lecturers

Edwin J. Cohn, Associate Professor of Physical Chemistry, Harvard University.
Henry J. Fry, Associate Professor of Biology, Washington Square College New York University.

E. Newton Harvey, Professor of Physiology, Princeton University.
Selig Hecht, Professor of Biophysics, Columbia University.
Merkel H. Jacobs, Professor of General Physiology, University of Pennsylvania.
Balduin Lucké, Associate Professor of Pathology, University of Pennsylvania.

BOTANY

I. Investigation

C. E. Allen, Professor of Botany, University of Wisconsin.
S. C. Brooks, Professor of Zoölogy, University of California.
B. M. Duggar, Professor of Physiological and Economic Botany, University of Wisconsin.
Ivey F. Lewis, Professor of Biology, University of Virginia.
Wm. J. Robbins, Professor of Botany, University of Missouri.

II. Instruction

William Randolph Taylor, Professor of Botany, University of Pennsylvania.
James P. Poole, Professor of Evolution, Dartmouth College.
G. W. Prescott, Assistant Professor of Biology, Albion College.

LIBRARY

Priscilla B. Montgomery (Mrs. Thomas H. Montgomery, Jr.), Librarian.
Deborah Lawrence, Secretary.
Hazel Blanchard, Doris Endrejat, Mary A. Rohan, Assistants.

DEPARTMENT OF EXPERIMENTAL RADIOLOGY

G. Failla, Physicist, Memorial Hospital, New York City.

CHEMICAL SUPPLIES

Oscar W. Richards, Instructor in Biology, Yale University.

SCIENTIFIC APPARATUS AND TECHNICAL SUPPLIES

Samuel E. Pond, Assistant Professor of Physiology, Schools of Medicine and Dentistry, University of Pennsylvania, in charge.

A. R. Apgar, Photographer.
J. D. Graham, Glassblower.
Lester F. Boss, Mechanician.
P. H. Liljestrand, Assistant.

MUSEUM

George M. Gray, Curator.

SUPPLY DEPARTMENT

James McInnis, Manager.
A. M. Hilton, Collector.
Milton B. Gray, Collector.
Walter Kahler, Collector.
Geoffrey Lehy, Collector.
A. W. Leathers, Shipping.

BOATS

JOHN J. VEEDER, Captain. E. M. LEWIS, Chief Engineer.

F. M. MACNAUGHT, Business Manager.
THOMAS LARKIN, Superintendent of Mechanical Department.
WILLIAM HEMENWAY, Carpenter.

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2. INVESTIGATORS AND STUDENTS, 1932

Independent Investigators

ADDISON, WILLIAM H. F., Professor of Normal Histology and Embryology, University of Pennsylvania, School of Medicine.
AMBERSON, WILLIAM R., Professor of Physiology, University of Tennessee.
ANDERSON, RUBERT S., Research Associate, Princeton University.
ARMSTRONG, PHILIP B., Assistant Professor of Anatomy, Cornell University Medical College.
BAITSELL, GEORGE A., Professor of Biology, Yale University.
BAKER, HORACE B., Associate Professor, University of Pennsylvania.
BALLARD, WILLIAM W., Instructor in Zoölogy, Dartmouth College.
BARD, PHILIP, Assistant Professor of Physiology, Harvard Medical School.
BARTH, L. G., Instructor, Columbia University.
BEAMS, H. W., Assistant Professor of Zoölogy, State University of Iowa.
BELKIN, MORRIS, Instructor, New York University.
BIGELOW, ROBERT P., Professor of Zoölogy and Parasitology, Massachusetts Institute of Technology.
BISSONNETTE, T. HUME, Professor of Biology, Trinity College.
BODANSKY, AARON, Research Biochemist, Hospital for Joint Diseases, New York.
BORODIN, DMITRY N., Yonkers, New York.
BOWLING, RACHEL, Instructor in Zoölogy, Columbia University.
BOYER, DONALD A., Instructor, the University of Chicago.
BOZLER, EMIL, Fellow in Medical Physics, Johnson Foundation for Medical Physics.
BRIDGES, CALVIN B., Research Assistant, Carnegie Institution of Washington.
BRINLEY, FLOYD J., Assistant Professor of Zoölogy, North Dakota State College.
BROOKS, M. M., Research Associate, University of California.
BROOKS, S. C., Professor of Zoölogy, University of California.
BROWN, DUGOLD E. S., Instructor in Physiology, New York University.
BUCHSBAUM, RALPH, Instructor, the University of Chicago.
BUDINGTON, ROBERT A., Professor of Zoölogy, Oberlin College.
CABLE, RAYMOND M., Teaching Fellow, New York University.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CALLOW, BESSIE R., In Charge of Rheumatic Fever Laboratory, New York University, Medical College.
CAROTHERS, E. ELEANOR, Lecturer in Zoölogy, University of Pennsylvania.
CARPENTER, RUSSELL L., Associate in Anatomy, College of Physicians and Surgeons, Columbia University.
CASTLE, WILLIAM A., Instructor in Biology, Brown University.
CATTELL, WARE, Garrison, New York.
CHAMBERS, ROBERT, Research Professor, Washington Square College, New York University.
CHANUTIN, ALFRED, Professor of Biochemistry, University of Virginia.
CHENEY, RALPH H., Chairman, Biology Department, Long Island University.
CHIDESTER, F. E., Professor of Zoölogy, West Virginia University.

CLARK, ELEANOR LINTON, University of Pennsylvania.
CLARK, ELIOT R., Professor of Anatomy, University of Pennsylvania.
CLOWES, GEORGE H. A., Director of Research, Lilly Research Laboratories.
COE, WESLEY R., Professor of Biology, Yale University.
COHEN, ROSE S., Graduate Assistant in Zoölogy, University of Cincinnati.
COLE, ELBERT C., Associate Professor of Biology, Williams College.
COLE, KENNETH S., Assistant Professor of Physiology, Columbia University.
COLE, ROBERT H., Oberlin College.
CONKLIN, EDWIN G., Professor of Biology, Princeton University.
COONFIELD, B. R., Instructor in Biology, Brooklyn College.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COWLES, R. P., Professor of Zoölogy, Johns Hopkins University.
CROASDALE, HANNAH T., Assistant in Botany, University of Pennsylvania.
DARLINGTON, C. D., Cytologist, John Innes Horticultural Institution, England.
DAWSON, ALDEN B., Associate Professor of Zoölogy, Harvard University.
DONALDSON, HENRY H., Member, Wistar Institute.
DREYER, WILLIAM A., Instructor in Zoölogy, University of Cincinnati.
DURYEE, WILLIAM R., Instructor, Department of Zoölogy, Northwestern University.
EDWARDS, DAYTON J., Associate Professor of Physiology, Cornell University Medical College.
EINARSON, LARUS, Research Fellow of the Rockefeller Foundation, Johns Hopkins University Medical School.
FLEISHER, MOYER S., Professor of Bacteriology and Hygiene, St. Louis University, School of Medicine.
FRASER, DORIS A., Assistant in Anatomy, University of Pennsylvania, Medical School.
FRY, HENRY J., Professor of Biology, Washington Square College, New York University.
GARREY, W. E., Professor of Physiology, Vanderbilt University School of Medicine.
GERARD, R. W., Associate Professor of Physiology, the University of Chicago.
GLASER, O. C., Professor, Amherst College.
GLASER, R. W., Associate Member, Rockefeller Institute for Medical Research.
GOLDFORB, A. J., Professor of Biology, College of the City of New York.
GOLDSCHMIDT, RICHARD B., Director, Kaiser-Wilhelm-Institut für Biologie, Berlin-Dahlem, Germany.
GOLDSMITH, E. D., Assistant in Zoölogy, Harvard University.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GOULD, R. GORDON, JR., Tutor in Bio-chemical Sciences, Harvard University.
GRAVE, B. H., Professor of Zoölogy, DePauw University.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
GREEN, DAVID E., Assistant, Washington Square College.
GUERLAC, HENRY E., Assistant in Physiology, Cornell University.
HADLEY, CHARLES E., Assistant Professor of Biology, New Jersey State Teachers College at Montclair.
HAHNERT, WILLIAM F., National Research Fellow in Biological Sciences, Johns Hopkins University.
HARNLY, MORRIS H., Assistant Professor, Washington Square College, New York University.
HARVEY, ETHEL B., Research Investigator, Princeton University.
HARVEY, E. N., Professor of Physiology, Princeton University.
HEILBRUNN, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
HENSHAW, P. S., Biophysicist, Memorial Hospital, New York City.
HERRICK, EARL H., Professor of Biology and Head of Department, Louisiana State Normal College.
HILL, SAMUEL E., Assistant in General Physiology, Rockefeller Institute.
HOADLEY, LEIGH, Professor of Zoölogy, Harvard University.

HODGE, CHARLES, JR., Instructor in Zoölogy, University of Pennsylvania.
HOOK, SABRA J., Assistant Professor of Biology, University of Rochester.
HOPPE, ELLA N., Research Assistant in Biology, New York State Department of Health.
HOWE, H. E., Editor, Industrial and Engineering Chemistry.
HOWLAND, RUTH B., Associate Professor of Biology, Washington Square College, New York University.
HUETTNER, ALFRED F., Professor of Biology, Washington Square College, New York University.
HULL, FRANK M., Head of Department of Biology, University of Mississippi.
HUNT, THOMAS E., Assistant Professor of Anatomy, University of Alabama, School of Medicine.
IRVING, LAURENCE, Associate Professor of Physiology, University of Toronto.
JACKSON, J. R., Graduate Assistant in Botany, University of Missouri.
JACOBS, MERKEL H., Professor of General Physiology, University of Pennsylvania.
JAHN, THEO. L., Fellow, National Research Council.
JENKINS, GEORGE B., Professor and Director Department of Anatomy, George Washington University.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt University, School of Medicine.
JOHNSTON, JANET L., 250 S. 21st Street, Philadelphia, Pennsylvania.
KAUFMANN, BERWIND P., Professor of Botany, University of Alabama.
KEIL, ELSA M., Instructor in Zoölogy, New Jersey College for Women.
KERR, THOMAS, National Research Fellow, University of Pennsylvania.
KEYES, D. B., Professor of Industrial Chemistry, University of Illinois.
KIDDER, GEORGE W., Tutor, College of the City of New York.
KIRKPATRICK, T. BRUCE, Associate Professor of Physical Education, Columbia University.
KLEIN, HENRY, Research Fellow, Johns Hopkins University.
KNOWER, HENRY MCE., Associate Professor of Anatomy, Albany Medical College.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, College of Medicine.
KRIEG, WENDELL J. S., Instructor in Anatomy, New York University.
LACAILLADE, CHARLES WM., Fellow, The Rockefeller Institute.
LACKEY, JAMES B., Professor of Biology, Southwestern.
LANCEFIELD, DONALD E., Associate Professor of Zoölogy, Columbia University.
LANCEFIELD, REBECCA C., Associate in Bacteriology, Rockefeller Institute.
LAUG, EDWIN P., Instructor in Physiology, University of Pennsylvania.
LEVY, MILTON, Instructor, University and Bellevue Hospital Medical College.
LEWIS, IVEY F., Professor of Botany, University of Virginia.
LILLIE, FRANK R., Chairman of the Department of Zoölogy, the University of Chicago.
LILLIE, RALPH S., Professor of General Physiology, the University of Chicago.
LINDERSTROM-LANG, KAJ. M., Inspector, Carlsberg Laboratorium.
LIPMANN, FRITZ, Associate, Rockefeller Foundation.
LOEFER, JOHN B., University Fellow, University College, New York University.
LYNCH, RUTH STOCKING, Instructor, Johns Hopkins University.
MCGOUN, RALPH C., JR., Instructor in Biology, Amherst College.
MCGREGOR, JAMES H., Professor of Zoölogy, Columbia University.
MACHLIS, SAMUEL, Assistant in Biology, Washington Square College, New York University.
MAGRUDER, SAMUEL R., Graduate Assistant in Zoölogy, University of Cincinnati.
MARTIN, EARL A., Chairman of Biology Department, Brooklyn College.
MAST, S. O., Professor of Zoölogy, Johns Hopkins University.
MATHEWS, ALBERT P., Carnegie Professor of Biochemistry and Head of Department, University of Cincinnati.

MATTHEWS, SAMUEL A., Associate in Anatomy, University of Pennsylvania.
MAZIA, DANIEL, Undergraduate Student, University of Pennsylvania.
MICHAELIS, LEONOR, Member, Rockefeller Institute for Medical Research.
MILLER, FORREST W., Graduate Assistant, University of Pittsburgh.
MONTALENTI, GIUSEPPE, Assistant in the Zoölogical Institute, Rome University, Rome, Italy.
MOORE, A. R., Research Professor of Physiology, University of Oregon.
MORGAN, LILIAN V., California Institute of Technology.
MORGAN, T. H., Professor of Biology, California Institute of Technology.
MORRILL, CHARLES V., Associate Professor of Anatomy, Cornell University Medical College.
NELSEN, OLIN E., Instructor in Zoölogy, University of Pennsylvania.
NELSON, E. CLIFF, Student Assistant, Johns Hopkins School of Hygiene and Public Health.
NONIDEZ, JOSÉ F., Assistant Professor of Anatomy, Cornell University Medical College.
ORIAS, OSCAR, Fellow, Rockefeller Foundation.
OSTERHOUT, W. J. V., Member, Rockefeller Institute for Medical Research.
PACKARD, CHARLES, Assistant Professor of Zoölogy, Columbia University.
PALMER, ALBERT H., New York University Medical College.
PAPENFUSS, G. F., Graduate Student, Johns Hopkins University.
PARKER, G. H., Professor of Zoölogy, Harvard University.
PARPART, ARTHUR K., Instructor in Physiology, Princeton University.
PAYNE, FERNANDUS, Professor of Zoölogy, Indiana University.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
POLLISTER, ARTHUR W., Instructor in Zoölogy, Columbia University.
POLLISTER, PRISCILLA FREW, Graduate Student, Columbia University.
POND, SAMUEL E., Assistant Professor of Physiology, University of Pennsylvania, Medical School.
POOLE, JAMES P., Professor of Evolution, Dartmouth College.
POTTER, TRUMAN S., Seymour Coman Fellow in Preventive Medicine, University of Chicago.
PRESCOTT, G. W., Assistant Professor of Biology, Albion College.
DE RENYI, GEORGE S., Associate Professor of Anatomy, University of Pennsylvania.
REZNIKOFF, PAUL, Instructor in Medicine, Cornell University Medical College.
RICHARDS, OSCAR W., Instructor in Biology, Yale University.
ROBBINS, WILLIAM J., Professor of Botany, University of Missouri.
ROBERT, NAN L., Instructor in Zoölogy, Hunter College.
ROOT, WALTER S., Associate Professor of Physiology, College of Medicine, Syracuse University.
RUGH, ROBERTS, Instructor in Zoölogy, Hunter College.
SAEGER, ALBERT C., National Research Fellow in the Biological Sciences, National Research Council.
SAYLES, LEONARD P., Instructor in Biology, College of the City of New York.
SCHAUFFLER, WILLIAM G., Princeton, New Jersey.
SCHERP, HENRY W., Assistant, Rockefeller Institute for Medical Research.
SCHMIDT, IDA T. GENTHER, Junior Research Fellow, Research Foundation of the Children's Hospital, Cincinnati.
SCHMIDT, LEON H., Research Fellow, University of Cincinnati, College of Medicine.
SCHMIEDER, RUDOLF G., Instructor in Zoölogy, University of Pennsylvania.
SCHOPPER, E. WERNER, Pathological Institute, Giessen, Germany.
SCHRADER, FRANZ, Professor of Zoölogy, Columbia University.
SCHRADER, SALLY HUGHES, Instructor in Zoölogy, Sarah Lawrence College.
SCOTT, SISTER FLORENCE MARIE, Assistant Professor, Seton Hill College.
SEVERINGHAUS, AURA E., Assistant Professor of Anatomy, Columbia University.
SHUMWAY, WALDO, Professor of Zoölogy, University of Illinois.

SILVEY, J. K. GWYNN, Instructor, University of Michigan.
SONNEBORN, TRACY M., Research Associate, Johns Hopkins University.
SPEICHER, B. R., Graduate Assistant, University of Pittsburgh.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia.
STABLER, ROBERT M., Instructor in Zoölogy, University of Pennsylvania.
STANLEY, WENDELL M., Research Assistant, Rockefeller Institute for Medical Research.
STEINBACH, HENRY BURR, Instructor in Zoölogy, University of Pennsylvania.
STEWART, DOROTHY R., Assistant Professor of Biology, Skidmore College.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell University Medical College.
STOKEY, ALMA G., Professor of Botany, Mount Holyoke College.
STREET, SIBYL F., Graduate Student, the University of Chicago.
STURTEVANT, A. H., Professor of Genetics, California Institute of Technology.
SUMWALT, MARGARET, Assistant Instructor, University of Pennsylvania, Medical School.
SUN, TSON P., Student, University of Pennsylvania.
TANG, PEI-SUNG, Instructor in General Physiology, Harvard University.
TASHIRO, SHIRO, Professor of Biochemistry, University of Cincinnati, College of Medicine.
TAYLOR, G. WELLFORD, Fellow in Biology, Princeton University.
TAYLOR, W. R., Professor of Botany, University of Michigan.
TITUS, CHARLES P., East Orange, New Jersey.
VICARI, EMILIA M., Associate in Anatomy, Cornell University Medical College.
WARREN, HOWARD C., Stuart Professor of Psychology, Princeton University.
WEISMAN, MAXWELL N., Graduate, Columbia University.
WEISS, PAUL A., Sterling Research Fellow, Research Institute of the Academy of Science in Vienna, Austria.
WENRICH, D. H., Professor of Zoölogy, University of Pennsylvania.
WHEDON, ARTHUR D., Professor of Zoölogy and Head of Department, North Dakota State College.
WHITING, ANNA R., Professor of Biology, Head of Department, Pennsylvania College for Women.
WHITING, P. W., Professor of Zoölogy, University of Pittsburgh.
WIEMAN, H. L., Professor of Zoölogy, University of Cincinnati.
WILLIER, BENJAMIN H., Professor of Zoölogy, the University of Chicago.
WILSON, EDMUND B., Professor Emeritus, Columbia University.
WINSOR, AGNES A., Volunteer Associate, Johns Hopkins University.
WINSOR, CHARLES P., Associate in Biology, Johns Hopkins University.
WITSCHI, EMIL, Professor of Zoölogy, State University of Iowa.
WOLF, E. ALFRED, Associate Professor of Zoölogy, University of Pittsburgh.
WOODRUFF, LORANDE LOSS, Professor of Protozoölogy, Yale University.
YOUNG, ROGER ARLINER, Assistant Professor of Zoölogy, Howard University.
ZELENY, CHARLES, Professor of Zoölogy, University of Illinois.
ZIRKLE, CONWAY, Associate Professor, University of Pennsylvania.

Beginning Investigators

ASHLEY, ALTA, Research Assistant, University of Cincinnati and Children's Hospital Research Foundation.
ATLAS, MEYER, Assistant in Zoölogy, Columbia University.
BELTRAN, ENRIQUE, Professor of Zoölogy, University of Mexico.
BRADBURY, HESTER ANN, Hardwick, Massachusetts.
BRIDGES, JOHN C., Graduate Student, Morehouse College.
BURR, EDITH ROGERS, Assistant in Zoölogy, Barnard College.
BUTLER, THOMAS, Student, Vanderbilt Medical School.
CAMPBELL, DAN H., Laboratory Assistant, Washington University.
CARLSON, J. GORDON, Instructor in Biology, Bryn Mawr College.

CARSON, RACHEL L., Graduate Student, Johns Hopkins University.
CHAO, IPING, Graduate Student, the University of Chicago.
CHEN, T. T., Instructor, University of Pennsylvania.
CHILD, GEORGE P., Assistant in Biology, New York University.
CHURCH, CHARLES F., Assistant Instructor in Pediatrics, University of Pennsylvania Medical School.
CLARK, JEAN M., Graduate Student, University of Pennsylvania.
CORSON, SAMUEL A., Research Assistant, Graduate Student, University of Pennsylvania.
COSTELLO, DONALD P., Instructor in Zoölogy, University of Pennsylvania.
CRUMMY, PRESSLEY L., Graduate Assistant, University of Pittsburgh.
DAN, KATSUMA, Graduate Student, University of Pennsylvania.
DIETER, CLARENCE D., Assistant Professor of Biology, Washington and Jefferson College.
EASTLICK, HERBERT L., Graduate Assistant in Zoölogy, Washington University.
FENNELL, RICHARD A., Assistant Professor, Birmingham College.
FISH, HAROLD S., Student and Assistant in Zoölogy, Harvard University.
FUCHS, W. BARRETT, Student Assistant in Biology, American University.
GRAND, C. G., Research Assistant, Washington Square College, New York University.
HENSHAW, CHRISTINE T., Assistant Biophysicist, Memorial Hospital, New York City.
HITCHCOCK, HAROLD B., Assistant, Williams College.
HODGE, RUTH PATRICK, Graduate Student, University of Virginia.
KALISS, NATHAN, Assistant in Zoölogy, Columbia University.
KANRICH, DOROTHY M., Graduate Student in Physiology and Instructor, Carnegie Institute of Technology, Pittsburgh, Pennsylvania.
KELTCH, ANNA K., Eli Lilly Research Laboratory.
KEMPTON, RUDOLF T., Instructor, New York University.
KEOSIAN, JOHN, Assistant in Biology, Washington Square College, New York University.
KIESS, MARY D., University of Pennsylvania.
KILLE, FRANK R., Graduate Student, University of Chicago.
KINNEY, ELIZABETH T., Lecturer in Zoölogy, Barnard College.
LANDOWNE, MILTON, Fellow in Biology, College of the City of New York.
LAWLOR, SISTER ANNA CATHERINE, Instructor in Biology, College of Saint Elizabeth.
L'HERITIER, PHILIPPE, Rockefeller Fellow, École Normale Supérieure, Paris, France.
LUNDSTROM, HELEN M., Chemist, Children's Hospital of Philadelphia.
MARSLAND, DOUGLAS A., Assistant Professor of Biology, Washington Square College, New York University.
METZNER, JEROME, Graduate Student, Columbia University.
MIHALIK, PETER, Assistant Professor, University of Budapest.
MODELL, WALTER, Assistant in Anatomy, Cornell University Medical College.
MORRIS, JOHN E., Graduate Student and Laboratory Assistant, Fisk University.
MOSELEY, JOHN E., Student of Biology, Harvard College.
NICOLL, PAUL A., Assistant in Zoölogy, Washington University.
ORMSBY, ANDREW A., Research Assistant, Pennsylvania State College.
PANDIT, C. G., Assistant Director, King Institute of Preventive Medicine, Madras, India.
PIERCE, MADELENE E., Instructor in Zoölogy, Vassar College.
PIERSON, BERNICE F., Graduate Student, Johns Hopkins University.
PROSSER, C. LADD, Graduate Student, Johns Hopkins University.
ROWLAND, CLAUDE R., Assistant in Zoölogy, Columbia University
RUNDLES, WAYNE, Undergraduate Assistant, DePauw University.

RUSSELL, HENRY D., Student, Harvard University.
SCHECHTER, VICTOR, Tutor in Department of Biology, College of the City of New York.
SCOTT, ALLAN C., Assistant in Zoölogy, Columbia University.
SHAPIRO, HERBERT, Assistant in Biology, Princeton University.
SICHEL, FERDINAND J. M., Assistant in Biology, Washington Square College, New York University.
SMITH, BRUCE A., Graduate Student, University of Pittsburgh.
SMITH, EMIL L., Assistant in Zoölogy, Columbia University.
SOUTHWICK, WALTER E., Austin Teaching Fellow, Harvard University.
SPECHT, HEINZ, Graduate Student, Johns Hopkins University.
STURDIVANT, HARWELL P., Assistant Professor of Biology, Emory University.
SUMMERS, FRANCIS M., Columbia University.
TAYLOR, GEORGIANA, Graduate Student in Zoölogy, University of Pittsburgh.
TEWINKEL, LOIS E., Graduate Student, Columbia University.
THOMAS, THURLO B., Graduate Assistant, Oberlin College.
TITTLER, IRVING A., Assistant in Zoölogy, Columbia University.
TOWNSEND, GRACE, Fellow, the University of Chicago.
WALKER, PAUL A., Graduate Student, Harvard University.
WILSON, HILDEGARD, Teaching Fellow, University and Bellevue Hospital, New York University, Medical College.
WINOKUR, MORRIS, Fellow, College of the City of New York.

Research Assistants

ALDERMAN, EVANGELINE, Instructor, Wellesley College.
BECK, LYLE V., Graduate Assistant, Department of Zoölogy, University of Pittsburgh.
BUTT, CHARLES, Research Assistant, Princeton University.
CHUTE, A. LAURENCE, Fellow in Physiology, University of Toronto.
CRAMPTON, CLAIR B., Instructor, Wesleyan University.
DOYLE, WILLIAM L., Assistant in Zoölogy, Johns Hopkins University.
DRIGGS, MARSHALL F., Medical Student, Cornell University Medical College.
DUNN, EDWIN E., Research Assistant, University of Cincinnati.
EYRE, SARAH W., Research Assistant, Long Island University.
GILMORE, KATHRYN, Technical Assistant, University of Pittsburgh.
GOODSON, MARY L., Student, Barnard College.
GRAHAM, C. H., Research Assistant, University of Pennsylvania.
HARNLY, MARIE L., Assistant in Biology, Washington Square College, New York University.
HARRYMAN, ILENE, Lilly Research Laboratories.
HILL, EDGAR S., Research Assistant, Rockefeller Institute.
MICHAELIS, EVA, Research Assistant, Columbia University.
MILLS, SYLVIA M., Research Assistant, Radcliffe College.
MORELAND, FERRIN B., Graduate Assistant, Vanderbilt University.
PACE, DONALD M., Research Assistant, General Physiology, Johns Hopkins University.
PARKS, ELIZABETH K., Instructor in Histology and Embryology, Boston University School of Medicine.
PARKS, MARK E., Assistant Instructor in Biology, New York University.
PORTER, HELEN, Research Assistant, Harvard University.
RAWLES, MARY E., Research Assistant, the University of Chicago.
ROBERTSON, C. W., Assistant in Biology, New York University.
SANGER, GRANT, Medical Student, Cornell University Medical College.
SELL, JAMES P., Laboratory Assistant, Yale University.
STARKEY, WILLIAM F., Graduate Student in Zoölogy, University of Pittsburgh.
WADE, LUCILLE W., Eli Lilly Research Laboratories.
YOUNG, SAUL B., Technician, Rockefeller Institute for Medical Research.

Students

BOTANY

BACH, DORIS A., University of Michigan.
BRUBAKER, ETHEL, Teacher of Biology, Philadelphia High School.
GUSTAFSON, ALTON H., Instructor in Biology, Williams College.
LAWLOR, JOHN T., JR., Austin Teaching Fellow, Harvard University.
MACARTHUR, MARY, Student, Acadia University.
MARQUETTE, WILLIAM G., Student, Columbia University.
PRIMROSE, HELEN L., Hunter College.
RUSSELL, DOROTHY M., Pennsylvania College for Women.
SPANGLER, ELIZABETH A., Wheaton College.
STRONGMAN, LOUISE E., Student, Radcliffe College.
WEBSTER, MARGARET D., Dalhousie University.
WEINTRAUB, ROBERT L., George Washington University.
WILHELM, HELEN M., Hunter College.
WISMER, VIRGINIA, Assistant in Botany, University of Pennsylvania.

EMBRYOLOGY

AIKEN, ROBERT B., Research Fellow, University of Vermont.
BELCHER, JANE C., Colby College.
BRADBURY, HESTER A., Hardwick, Massachusetts.
BURROWS, ROBERT B., JR., Assistant Graduate Student, Yale University.
COWLES, JANET M., Johns Hopkins University.
CUMMINGS, FRANCES, 122 Elm Street, Meriden, Connecticut.
DEE, JOHN E., Wesleyan University.
DIETER, CLARENCE D., Assistant Professor of Biology, Washington and Jefferson College.
EASTLICK, HERBERT L., Graduate Assistant in Zoölogy, Washington University.
HEISS, MARY E., Wellesley College.
HOOVER, MARGARET E., Student, Smith College.
HUSSEY, KATHLEEN L., Fellow in Zoölogy, Ohio Wesleyan University.
KLEINHOLZ, LEWIS H. K., Instructor in Anatomy, Colby College.
KUGLER, OTTO E., Tutor, Illinois University.
LEWIS, RALPH H., Assistant in Biology, University of Rochester.
MILLER, DOROTHY K., Bryn Mawr College.
MORRIS, JOHN E., Laboratory Assistant in Biology, Fisk University.
OLSEN, MARLOW W., Junior Poultry Biologist, United States Department of Agriculture.
PENN, AMOS B. K. C., Graduate Student, Johns Hopkins University.
PFEIFER, KATHERINE, Teacher at Soldan High School, St. Louis.
POMERAT, C. M., Assistant in Biology, Clark University.
RUNDLES, RALPH W., DePauw University.
SCATTERTY, LOUISE E., Instructor, H. Sophie Newcomb College.
SIMS, D. LAWRENCE, Student, DePauw University.
SMITH, VERA I., Teaching Fellowship, Brown University.
SULLIVAN, JOHN L., JR., Johns Hopkins University.
WAGONER, KENNETH S., DePauw University.
WATKEYS, JEAN DE G., Student, University of Rochester.
WIRTZ, SISTER ST. MARK, Instructor in Biology, College of St. Catherine.

PHYSIOLOGY

BRIDGES, JOHN C., Instructor, Morehouse College.
BUTLER, THOMAS C., Student, Vanderbilt Medical School.
CHAO, IPING, the University of Chicago.

COPLAN, HELEN M., Assistant in Biology, Goucher College.
CRAIG, FRANCIS N., Rutgers University.
HESS, MARGARET, University of Virginia.
HEYL, JAMES T., Hamilton College.
KING, FLORICE A., Graduate Assistant in Physiology, Wellesley College.
McINTIRE, JOSEPHINE M., Assistant, Mount Holyoke College.
MANERY, JEANNE F., Student, University of Toronto.
PAPPENHEIMER, ANNE, Radcliffe College.
RIEDMAN, SARAH R., Instructor, Department of Physiology, Brooklyn College.
ROEDER, KENNETH D., Instructor in Physiology, Tufts College.
SCHOTT, MARGARET, Assistant, Mount Holyoke College.
SPECHT, HEINZ, Graduate Student, Johns Hopkins University.
TOOTHILL, MARTHA C., Instructor in General Biology, Adelphi College.
TUM-SUDEN, CAROLINE, Research Fellow, Boston University.
WARBRITTON, VIRGENE, Research Assistant, University of Missouri.

PROTOZOÖLOGY

BELTRAN, ENRIQUE, Professor of Zoölogy, University of Mexico.
BINGHAM, N. ELDRED, Instructor, Montclair State Teachers College.
BROWN, REBECCA, Goucher College.
DUNCAN, PAUL M., University of Pennsylvania.
EARL, RUTH R., Graduate Student, New York University.
FUCHS, WILLIAM B., American University.
KELLY, FLORENE CORA, Instructor, Simmons College.
LAWLOR, SISTER ANNA CATHERINE, Instructor in Biology, College of Saint Elizabeth.
LEVIN, ANNA C., Columbia University.
LING, SHAO-WEN, Graduate Student, The Rockefeller Foundation.
McDONALD, CLARA M., Nassau Hospital, Minneola.
MANTER, JOHN T., Student Assistant, Columbia University.
METZNER, JEROME, College of the City of New York.
ROWLAND, CLAUDE R., Columbia University.
SMITH, THORN, JR., Student, Columbia University.
WILLIS, DORIS M., American University.

INVERTEBRATE ZOÖLOGY

ALDINGER, LENORE, University of Wisconsin.
ANTHONY, GENEVIEVE, Graduate Student, University of Pennsylvania.
AXFORD, DOROTHY, Graduate Assistant, New Jersey College for Women.
BATES, M. NOBLE, Graduate Assistant in Zoölogy, Hamilton College.
BELDING, HARWOOD, Laboratory Assistant, Connecticut State Agricultural College.
BERKENFELD, CHARLOTTE G., Teacher in Elementary Grades, Brooklyn, New York.
BUCHHEIT, J. ROBERT, Graduate Assistant in Zoölogy, University of Illinois.
COUCH, MARY L., Elmira College.
CROOKS, KENNETH B. M., Instructor in Biology, Hampton Institute.
DIACK, MARION, Student, Oberlin College.
DIBBLE, UEL E., Graduate Student, Yale University.
ELLIOTT, ALFRED M., Teaching Fellow, New York University.
FOLTZ, RUTH G., Student, Oberlin College.
GOFFIN, CATHERINE E., Student, Brown University.
GRAY, BEATRICE, Student, State University of Iowa.
GRIERSON, MARGARET C., Graduate Student, Mount Holyoke College.
HAFFNER, WAYNE, Wabash College.
HAMILTON, MARY A., Student, Elmira College.
HAVEY, CLINTON B., Student, Acadia University.

HENDERSON, RUTH E., Goucher College.
HOOVER, W. KENNETH, Laboratory Assistant, American University.
HUFF, GEORGE C., State University of Iowa.
IVES, PHILIP T., Amherst College.
JACQUES, RICHARD, Ohio Wesleyan University.
JONAS, MARION, New Jersey College for Women.
KLEINHOLZ, LEWIS H. K., Instructor, Colby College.
KOHN, HENRY I., Graduate Student, Yale University.
LARRABEE, MARTIN G., Harvard University.
LING, SHAO-WEN, Graduate Student, Cornell University.
LIPPY, GRACE E., Assistant Professor of Biology, Hood College.
LIVENGOOD, WAYNE F., Wabash College.
LOGAN, AMY D., Wilson College.
LUMER, HYMAN, Graduate Assistant, Western Reserve University.
MANUEL, BETH, Dalhousie University.
MEYER, ADELPHIA M., Student, Peabody College.
OLSEN, MARLOW W., Poultry Biologist, United States Department of Agriculture.
OWEN, CORA R., Student, Vassar College.
PENN, AMOS B. K. C., Graduate Student, Johns Hopkins University.
PLISKE, EDWARD C., Student, Oberlin College.
RANKIN, JOHN S., JR., Student, Wesleyan University.
REED, SHELDON C., Dartmouth College.
REES, OLIVE L., Assistant in Botany, University of Wisconsin.
SANDNES, GUNNAR C., College of the City of New York.
SCHLOEMER, CLARENCE L., Student, Beloit College.
SCHOENBORN, HENRY W., Student, DePauw University.
SETTY, LAUREL R., Instructor in Biology, Park College.
SHOEMAKER, HURST H., Instructor, Earlham College.
STEARNS, MARY L., Student, Smith College.
SURES, PEARL M., University of Minnesota.
TOBIAS, BELLE C., Instructor in Biology, Bennett College.
TUKEY, GERTRUDE R., Student, Smith College.
TURNER, ROBERT S., Student, Dartmouth College.
WARREN, MARSHALL R., Student, DePauw University.
WELLS, JOSEPHINE S., Barnard College.
ZINN, DONALD J., Student, Harvard University.

3. TABULAR VIEW OF ATTENDANCE

	1928	1929	1930	1931	1932
INVESTIGATORS—Total	323	329	337	362	314
Independent	217	234	217	236	212
Under Instruction	81	71	87	83	73
Research Assistants	25	24	33	43	29
STUDENTS—Total	133	125	136	125	132
Zoölogy	57	53	56	55	55
Protozoölogy	16	15	14	17	16
Embryology	29	28	27	29	29
Physiology	15	17	23	17	18
Botany	16	12	16	7	14
TOTAL ATTENDANCE	456	454	473	487	446
Less Persons registered as both Students and Investigators	2	10	14	20	14
	454	444	459	467	432

INSTITUTIONS REPRESENTED—Total	111	123	126	137	141
By Investigators	80	96	95	102	94
By Students	66	64	71	68	76
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	1	–	–	–	–
By Students	1	1	4	4	1
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	13	30	7	8	8
By Students	8	3	2	1	1

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS, 1932

Acadia University
Amherst College
Barnard College
Beloit University
Bowdoin College
Brown University
Bryn Mawr College
California Institute of Technology
Carnegie Institution of Washington
Chinese Educational Mission
College of St. Catherine
College of St. Elizabeth
Columbia University
Cornell University
Cornell University Medical College
Dalhousie University
Dartmouth College
DePauw University
Duke University
Elmira College
Fisk University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Johns Hopkins University
Eli Lilly & Co.
Long Island University
Memorial Hospital of New York City
Morehouse College
Mount Holyoke College
National Research Council
New York State Department of Health
New York University
New York University Medical School
Oberlin College
Pennsylvania College for Women
Princeton University
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Seton Hill College
Smith College
Sophie Newcomb College
Syracuse University
Tufts College
University of Chicago
University of Cincinnati
University of Illinois
University of Iowa
University of Missouri
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Vermont
University of Virginia
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Washington University
Wellesley College

Wesleyan University
Wheaton College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1932

Friday, July 1
Dr. Paul S. Galtsoff ..... "The Coral Reefs of the Hawaiian Islands."

Friday, July 8
Dr. R. W. Gerard ..... "The Speed of Life."

Friday, July 15
Dr. Rudolf Mond ..... "Regulation of Ions in the Body Tissues."

Friday, July 22
Dr. W. E. Garrey ..... "Some Aspects of the Physiology of the Heart of Limulus."

Monday, July 25
Dr. Leif Stoermer ..... "Were the Trilobites Related to Limulus?"

Friday, July 29
Dr. C. C. Speidel ..... "The Growth and Repair of Living Nerves."

Friday, August 5
Dr. R. Chambers ..... "The Vital Coloration of Protoplasm."

Friday, August 12
Dr. Alexander Forbes ..... "Surveying in Northern Labrador."

Friday, August 19
The Reynold A. Spaeth Memorial Lecture, delivered by Dr. R. Goldschmidt ..... "Genetics and Development."

Friday, August 26
Dr. H. H. Gran ..... "Problems in the Study of the Phytoplankton of the Sea."

SPECIAL LECTURES AND MOTION PICTURES

Wednesday, July 27
Dr. Paul Weiss (Motion Pictures) ..... "The Function of Supernumerary Limbs as Illustrating the Resonance Principle of Nervous Activity."

Thursday, August 18
Dr. A. R. Moore ..... "The Results of Prevention of Membrane Formation in Echinoderm Eggs."

Wednesday, August 24

Dr. W. Schopper ..... "Microcinematographs of Tissue Cultures of Organs from the Guinea Pig."

Friday, August 26

Dr. C. C. Speidel (Motion Pictures) ..... "The Growth and Repair of Living Nerves."

6. SHORTER SCIENTIFIC PAPERS, 1932

Tuesday, July 5

Dr. Ethel B. Harvey ..... "Splitting of the Eggs of Four Neapolitan Sea Urchins by Centrifugal Force and the Development of the Halves and Quarters."

Dr. Henry J. Fry and
Mr. Mark S. Parks ..... "The Relation between Viscosity Changes and Mitotic Changes in Cleaving Eggs."

Dr. L. V. Heilbrunn ..... "The Action of Ultra Violet Rays on the Protoplasm of Amœba."

Tuesday, July 12

Dr. Kenneth S. Cole ..... "Electric Phase Angle of Tissues."

Dr. Margaret Sumwalt,
Dr. W. R. Amberson and
Miss Eva Michaelis ..... "The Part Played by Diffusion Potentials in the Origin of Concentration Potential Differences across Frog Skin."

Dr. E. N. Harvey and
Dr. D. A. Marsland ..... "The Tension at the Surface of Amœba dubia."

Dr. E. N. Harvey ..... "The Beams Air Turbine for Biological Centrifuging."

Tuesday, July 19

Dr. A. B. Dawson ..... "The Relative Degrees of Differentiation of the Mature Erythrocytes of Vertebrates."

Dr. Paul Weiss ..... "The Factor which Determines the Orientation of the Growing Nerve Fiber."

Dr. G. H. Parker ..... "Neurohumoralism."

Dr. R. W. Gerard ..... "Observations on the Velocity of the Nerve Impulse."

Tuesday, July 26

Dr. M. M. Brooks ..... "Antagonism of Methylene Blue for CN and CO."

Dr. S. C. Brooks ..... "Partition Coefficients and Diffusion of Solutes in Heterogeneous Systems."

Dr. A. P. Mathews ..... "Nature of the Action of Enzymes."
Dr. Laurence Irving and
Mr. A. L. Chute ..... "The Participation of Bone in the Neutralization of Ingested Acid."

Tuesday, August 2
Dr. W. R. Taylor ..... "Phytoplankton of Isle Royale, Lake Superior."
Dr. Conway Zirkle ..... "Cytological Fixation with the Lower Fatty Acids, their Salts, etc."
Dr. G. W. Prescott ..... "Copper Sulphate as an Algacide in Lakes and Public Water Supplies."
Dr. Albert Saeger ..... "Manganese and the Growth of Lemnaceæ."

Tuesday, August 9
Mr. D. P. Costello ..... "Surface Precipitation Reaction in Marine Eggs."
Dr. P. S. Henshaw ..... "Changes in Sensitivity of Drosophila Eggs during Early Development to Hard and Soft X-rays, Gamma Rays and Alpha Particles."
Dr. R. M. Buchsbaum ..... "Size of Explant and Volume of Medium in Tissue Cultures."
Dr. B. H. Willier ..... "Germ Cells in Relation to the Origin and Differentiation of the Sex Gland of the Chick as Studied by Chorioallantoic Grafts."

Tuesday, August 16
Dr. Daniel Raffel ..... "Gene Mutations in Paramecium aurelia."
Dr. C. B. Bridges ..... "Chromosome Maps of Drosophila."
Dr. A. H. Sturtevant ..... "A New Unstable Translocation in Drosophila."

Tuesday, August 23
Dr. T. L. Jahn ..... "The Effects of Temperature and of Certain Organic Acid Radicals upon Euglena gracilis."
Mr. T. T. Chen ..... "Nuclear Structure and Mitosis in Zelleriella (Opalinidæ)."
Miss Sabra J. Hook ..... "Some Observations on Spirostomum ambiguum."
Dr. W. F. Hahnert ..... "Intensity Duration Relations in the Response of Certain Protozoa to the Electric Current."

Tuesday, August 30
Dr. A. W. Pollister ..... "The Development of Leucopoietic Tissue in Amblystoma punctatum."
Dr. W. H. F. Addison and
Dr. Doris A. Fraser ..... "Pigmentation in the Hypophysis and Parathyroids of the Gray Rat."

DR. GEORGE F. LAIDLAW ..... "The Dopa Reaction and the Problem of Pigment Formation in Mammalian Skin."

Friday, September 2

MR. C. M. GOSS,
MR. BRUCE HOGG AND
DR. KENNETH S. COLE ..... "Tissue Culture Action Potentials."
DR. ETHEL B. HARVEY ..... "Effects of Centrifugal Force on Fertilized Arbacia Eggs, as Observed with the Microscope Centrifuge."
DR. P. S. HENSHAW ..... "The Comparative Radiosensitivity of Marine Invertebrate Eggs."
DR. MARGARET SUMWALT ..... "Anomalous Potential Differences across Frog Skin."
DR. WALTER S. ROOT ..... "The Carbon Dioxide Dissociation Curve of Frog's Skeletal Muscle."
MR. S. A. CORSON ..... "The Effect of Acid and Alkali on the Plasmogel of Amœba proteus."
DR. F. J. BRINLEY ..... "The Action of Salts on Fundulus Embryos."
DR. OSCAR W. RICHARDS ..... "The Estimation of the Growth of Yeast Populations with a Photoelectric Cell."
DR. GEORGE A. BAITSELL ..... "A Simplified Technique for the Cultivation of Tissues in Vitro."
DR. T. M. SONNEBORN ..... "Some Genetic Consequences of Self-Fertilization and Cross-Fertilization in Paramecium aurelia."

DR. E. R. CLARK,
MRS. E. L. CLARK AND
DR. E. A. SWENSON ..... "Motion Pictures Showing the Contraction of Arterioles in the Rabbit's Ear."
DR. C. C. SPEIDEL ..... "Moving Pictures of the 'Fast Motion' Type of Various Cells in Living Frog Tadpoles."
MR. L. V. BECK ..... "The Effects of Penetrating and Non-Penetrating Acids and Bases on the Oxidation-Reduction Potential of Asterias Ova and of Asterias Sperm."

DR. G. H. A. CLOWES,
MISS ANNA K. KELTCH AND
MISS ILENE HARRYMAN ..... "On Inhibition of Maturation of Starfish Eggs by Acids and Acid Producing Agents and the Reversal of this Process by Alkalies."

Miss Anna K. Keltch,
Miss Lucille Wade, and
Dr. G. H. A. Clowes ..... "On the Contrasting Sensitivity of Eggs and Sperm to Various Chemical Agents."

Miss Ilene Harryman,
Miss Lucille Wade,
Miss Anna K. Keltch, and
Dr. G. H. A. Clowes ..... "On the Action of Soaps of the Oleate and Ricinoleate Series on Arbacia Sperm."

Dr. R. Chambers ..... "On the Formation of the Segmentation Furrow in the Sea Urchin Egg."

Dr. C. G. Pandit ..... "pH of the Arbacia Egg." (Presented by Dr. R. Chambers.)

Dr. Dorothy R. Stewart and
Dr. M. H. Jacobs ..... "The Influence of Temperature on the Permeability of the Arbacia Egg to Ethylene Glycol."

Dr. Dorothy R. Stewart and
Dr. M. H. Jacobs ..... "The Permeability of the Egg of Asterias to Water."

Mr. Otto Meier, Jr. ..... "The Use and Cost of Electrical Energy in Relation to Investigators in Attendance at the Marine Biological Laboratory."

7. MEMBERS OF THE CORPORATION

1. Life Members

Allis, Mr. E. P., Jr., Palais Carnoles, Menton, France.
Andrews, Mrs. Gwendolen Foulke, Baltimore, Md.
Billings, Mr. R. C., 66 Franklin St., Boston, Mass.
Conklin, Prof. Edwin G., Princeton University, Princeton, N. J.
Coolidge, Mr. C. A., Ames Building, Boston, Mass.
Crane, Mr. C. R., New York City.
Evans, Mrs. Glendower, 12 Otis Place, Boston, Mass.
Fay, Miss S. B., 88 Mt. Vernon St., Boston, Mass.
Foot, Miss Katherine, Care of Morgan Harjes Cie, Paris, France.
Gardiner, Mrs. E. G., Woods Hole, Mass.
Jackson, Miss M. C., 88 Marlboro St., Boston, Mass.
Jackson, Mr. Chas. C., 24 Congress St., Boston, Mass.
Kidder, Mr. Nathaniel T., Milton, Mass.
King, Mr. Chas. A.
Lee, Mrs. Frederic S., 279 Madison Ave., New York City, N. Y.
Lowell, Mr. A. Lawrence, 17 Quincy St., Cambridge, Mass.

McMurrich, Prof. J. P., University of Toronto, Toronto, Canada.
Means, Dr. James Howard, 15 Chestnut St., Boston, Mass.
Merriman, Mrs. Daniel, 73 Bay State Road, Boston, Mass.
Minns, Miss Susan, 14 Louisburg Square, Boston, Mass.
Morgan, Mr. J. Pierpont, Jr., Wall and Broad Sts., New York City, N. Y.
Morgan, Prof. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, Calif.
Morgan, Mrs. T. H., Pasadena, Calif.
Noyes, Miss Eva J.
Osborn, Prof. Henry F., American Museum of Natural History, New York, N. Y.
Phillips, Mrs. John C., Windy Knob, Wenham, Mass.
Porter, Dr. H. C., University of Pennsylvania, Philadelphia, Pa.
Sears, Dr. Henry F., 86 Beacon St., Boston, Mass.
Shedd, Mr. E. A.
Thorndike, Dr. Edward L., Teachers College, Columbia University, New York City, N. Y.
Trelease, Prof. William, University of Illinois, Urbana, Ill.
Ware, Miss Mary L., 41 Brimmer St., Boston, Mass.
Williams, Mrs. Anna P., 505 Beacon St., Boston, Mass.
Wilson, Dr. E. B., Columbia University, New York City, N. Y.

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Adams, Dr. A. Elizabeth, Mount Holyoke College, South Hadley, Mass.
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BAKER, DR. E. H., 5312 Hyde Park Boulevard, Hyde Park Station, Chicago, Ill.

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BRADLEY, PROF. HAROLD C., University of Wisconsin, Madison, Wis.

BRAILEY, MISS MIRIAM E., 800 Broadway, Baltimore, Md.

BRIDGES, DR. CALVIN B., California Institute of Technology, Pasadena, Calif.

BRONK, DR. D. W., University of Pennsylvania, Philadelphia, Pa.

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BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Va.

BUMPUS, PROF. H. C., 76 Carlton Road, Waban, Mass.

BYRNES, DR. ESTHER F., 1803 North Camac Street, Philadelphia, Pa.

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COHN, DR. EDWIN J., 183 Brattle Street, Cambridge, Mass.
COLE, DR. ELBERT C., Williams College, Williamstown, Mass.
COLE, DR. KENNETH S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.
COLE, DR. LEON J., College of Agriculture, Madison, Wis.
COLLETT, DR. MARY E., Western Reserve University, Cleveland, O.
COLLEY, MRS. MARY W., 36 Argyle Place, Rockville Centre, Long Island, N. Y.
COLTON, PROF. H. S., Box 127, Flagstaff, Ariz.
CONNOLLY, DR. C. J., Catholic University, Washington, D. C.
COONFIELD, DR. B. R., Brooklyn College, 80 Willoughby Street, Brooklyn, N. Y.
COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Me.
COWDRY, DR. E. V., Washington University, St. Louis, Mo.
CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City, N. Y.
CRANE, MRS. C. R., Woods Hole, Mass.
CURTIS, DR. MAYNIE R., Crocker Laboratory, Columbia University, New York City, N. Y.
CURTIS, PROF. W. C., University of Missouri, Columbia, Mo.
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DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Va.

DAWSON, DR. A. B., Harvard University, Cambridge, Mass.
DAWSON, DR. J. A., The College of the City of New York, New York City, N. Y.
DEDERER, DR. PAULINE H., Connecticut College, New London, Conn.
DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, W. Va.
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DONALDSON, PROF. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pa.
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FORBES, DR. ALEXANDER, Harvard University Medical School, Boston, Mass.
FRY, DR. HENRY J., Washington Square College, Washington Square, New York City, N. Y.
GAGE, PROF. S. H., Cornell University, Ithaca, N. Y.
GARREY, PROF. W. E., Vanderbilt University Medical School, Nashville, Tenn.
GATES, DR. F. L., 31 Fayerweather Street, Cambridge, Mass.
GATES, PROF. R. RUGGLES, University of London, London, England.
GEISER, DR. S. W., Southern Methodist University, Dallas, Tex.
GERARD, PROF. R. W., University of Chicago, Chicago, Ill.
GLASER, PROF. O. C., Amherst College, Amherst, Mass.
GOLDFORB, PROF. A. J., College of the City of New York, New York City, N. Y.
GOODRICH, PROF. H. B., Wesleyan University, Middletown, Conn.
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GREENMAN, PROF. M. J., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
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HARVEY, PROF. E. N., Princeton University, Princeton, N. J.
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HOOKER, PROF. DAVENPORT, University of Pittsburgh, Pittsburgh, Pa.
HOPKINS, DR. HOYT S., New York University, College of Dentistry, New York City, N. Y.
HOWE, DR. H. E., 2702 36th Street, N.W., Washington, D. C.
HOWLAND, DR. RUTH B., Washington Square College, New York University, Washington Square, East, New York City, N. Y.
HOYT, DR. WILLIAM D., Washington and Lee University, Lexington, Va.

HUMPHREY, MR. R. R., University of Buffalo, School of Medicine, Buffalo, N. Y.
HYMAN, DR. LIBBIE H., 41 West 70th Street, New York City, N. Y.
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KNAPKE, REV. BEDE, St. Bernard's College, St. Bernard, Ala.
KNOWER, PROF. H. McE., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.
KNOWLTON, PROF. F. P., Syracuse University, Syracuse, N. Y.
KOSTIR, DR. W. J., Ohio State University, Columbus, O.
KRIBS, DR. HERBERT, 202A Copley Road, Upper Darby, Pa.
KUYK, DR. MARGARET P., Westbrook Ave., Richmond, Va.
LANCEFIELD, DR. D. E., Columbia University, New York City, N. Y.
LANGE, DR. MATHILDE M., Wheaton College, Norton, Mass.
LEE, PROF. F. S., College of Physicians and Surgeons, New York City, N. Y.
LEWIS, PROF. I. F., University of Virginia, Charlottesville, Va.
LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Md.
LILLIE, PROF. FRANK R., The University of Chicago, Chicago, Ill.
LILLIE, PROF. RALPH S., The University of Chicago, Chicago, Ill.
LINTON, PROF. EDWIN, University of Pennsylvania, Philadelphia, Pa.
LOEB, PROF. LEO, Washington University Medical School, St. Louis, Mo.
LOWTHER, MRS. FLORENCE DEL., Barnard College, Columbia University, New York City, N. Y.

Lucké, Prof. Balduin, University of Pennsylvania, Philadelphia, Pa.
Lund, Dr. E. J., University of Texas, Austin, Tex.
Luscombe, Mr. W. O., Woods Hole, Mass.
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Lynch, Dr. Ruth Stocking, Johns Hopkins University, Baltimore, Md.
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MacDougall, Dr. Mary S., Agnes Scott College, Decatur, Ga.
McClung, Prof. C. E., University of Pennsylvania, Philadelphia, Pa.
McGee, Dr. Anita Newcomb, Box 363, Southern Pines, N. C.
McGregor, Dr. J. H., Columbia University, New York City, N. Y.
Macklin, Dr. Charles C., School of Medicine, University of Western Ontario, London, Canada.
Malone, Prof. E. F., University of Cincinnati, Cincinnati, O.
Manwell, Dr. Reginald D., Syracuse University, Syracuse, N. Y.
Martin, Prof. E. A., The College of the City of New York, New York City, N. Y.
Mast, Prof. S. O., Johns Hopkins University, Baltimore, Md.
Mathews, Prof. A. P., University of Cincinnati, Cincinnati, O.
Mavor, Prof. James W., Union College, Schenectady, N. Y.
Medes, Dr. Grace, University of Minnesota, Minneapolis, Minn.
Meigs, Dr. E. B., Dairy Division Experiment Station, Beltsville, Md.
Meigs, Mrs. E. B., 1736 M Street, N.W., Washington, D. C.
Metcalf, Prof. M. M., 94 Nehoiden Road, Waban, Mass.
Metz, Prof. Charles W., Johns Hopkins University, Baltimore, Md.
Michaelis, Dr. Leonor, Rockefeller Institute, New York City, N. Y.
Miller, Dr. Helen M., Yale University, New Haven, Conn.
Mitchell, Dr. Philip H., Brown University, Providence, R. I.
Moore, Dr. Carl R., The University of Chicago, Chicago, Ill.
Moore, Prof. George T., Missouri Botanical Garden, St. Louis, Mo.
Moore, Prof. J. Percy, University of Pennsylvania, Philadelphia, Pa.
Morgulis, Dr. Sergius, University of Nebraska, Lincoln, Nebr.
Morrill, Prof. A. D., Hamilton College, Clinton, N. Y.
Morrill, Prof. C. V., Cornell University Medical College, New York City, N. Y.
Muller, Dr. H. J., University of Texas, Austin, Tex.
Nabours, Dr. R. K., Kansas State Agricultural College, Manhattan, Kans.
Neal, Prof. H. V., Tufts College, Tufts College, Mass.
Newman, Prof. H. H., University of Chicago, Chicago, Ill.
Nichols, Dr. M. Louise, Rosemont, Pa.
Noble, Dr. Gladwyn K., American Museum of Natural History, New York City, N. Y.

NONIDEZ, DR. JOSÉ F., Cornell University Medical College, New York City, N. Y.

OKKELBERG, DR. PETER, University of Michigan, Ann Arbor, Mich.

OSBURN, PROF. R. C., Ohio State University, Columbus, O.

OSTERHOUT, PROF. W. J. V., Rockefeller Institute, New York City, N. Y.

PACKARD, DR. CHARLES, Columbia University, Institute of Cancer Research, 1145 Amsterdam Ave., New York City, N. Y.

PAGE, DR. IRVINE H., Rockefeller Institute, New York City, N. Y.

PAPANICOLAOU, DR. GEORGE N., Cornell University Medical College, New York City, N. Y.

PAPPENHEIMER, DR. A. M., Columbia University, New York City, N. Y.

PARKER, PROF. G. H., Harvard University, Cambridge, Mass.

PATON, PROF. STEWART, Princeton University, Princeton, N. J.

PATTEN, DR. BRADLEY M., Western Reserve University, Cleveland, O.

PATTEN, PROF. WILLIAM, Dartmouth College, Hanover, N. H.

PAYNE, PROF. F., University of Indiana, Bloomington, Ind.

PEARL, PROF. RAYMOND, Institute for Biological Research, 1901 East Madison Street, Baltimore, Md.

PEARSE, PROF. A. S., Duke University, Durham, N. C.

PEEBLES, PROF. FLORENCE, California Christian College, Los Angeles, Calif.

PHILLIPS, DR. E. F., Cornell University, Ithaca, N. Y.

PIKE, PROF. FRANK H., 437 West 59th Street, New York City, N. Y.

PINNEY, DR. MARY E., Milwaukee-Downer College, Milwaukee, Wis.

PLOUGH, PROF. HAROLD H., Amherst College, Amherst, Mass.

POLLISTER, DR. A. W., Columbia University, New York City, N. Y.

POND, DR. SAMUEL E., University of Pennsylvania, School of Medicine, Philadelphia, Pa.

PRATT, DR. FREDERICK H., Boston University, School of Medicine, Boston, Mass.

RAFFEL, DR. DANIEL, Zoölogical Laboratory, Johns Hopkins University, Baltimore, Md.

RAND, DR. HERBERT W., Harvard University, Cambridge, Mass.

RANKIN, PROF. W. M., Princeton University, Princeton, N. J.

REDFIELD, DR. ALFRED C., Harvard University, Cambridge, Mass.

REESE, PROF. ALBERT M., West Virginia University, Morgantown, W. Va.

REINKE, DR. E. E., Vanderbilt University, Nashville, Tenn.

DE RENYI, DR. GEORGE S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.

REZNIKOFF, DR. PAUL, Cornell University Medical College, New York City, N. Y.

RHODES, PROF. ROBERT C., Emory University, Atlanta, Ga.

RICE, PROF. EDWARD L., Ohio Wesleyan University, Delaware, O.

RICHARDS, PROF. A., University of Oklahoma, Norman, Okla.

RICHARDS, DR. O. W., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.

RIGGS, MR. LAWRASON, JR., 25 Broad Street, New York City, N. Y.

ROGERS, PROF. CHARLES G., Oberlin College, Oberlin, O.

ROMER, DR. ALFRED S., The University of Chicago, Chicago, Ill.

ROOT, DR. W. S., Syracuse Medical School, Syracuse, N. Y.

SAYLES, DR. LEONARD P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, N. Y.

SCHRADER, DR. FRANZ, Department of Zoölogy, Columbia University, New York City, N. Y.

SCHRADER, DR. SALLY HUGHES, Department of Zoölogy, Columbia University, New York City, N. Y.

SCHRAMM, PROF. J. R., University of Pennsylvania, Philadelphia, Pa.

SCOTT, DR. ERNEST L., Columbia University, New York City, N. Y.

SCOTT, PROF. G. G., College of the City of New York, New York City, N. Y.

SCOTT, PROF. JOHN W., University of Wyoming, Laramie, Wyo.

SCOTT, PROF. WILLIAM B., 7 Cleveland Lane, Princeton, N. J.

SEVERINGHAUS, DR. AURA E., Department of Anatomy, College of Physicians and Surgeons, 630 W. 168th Street, New York City, N. Y.

SHULL, PROF. A. FRANKLIN, University of Michigan, Ann Arbor, Mich.

SHUMWAY, DR. WALDO, University of Illinois, Urbana, Ill.

SIVICKIS, DR. P. B., Pasto deze 130, Kaunas, Lithuania.

SNOW, DR. LAETITIA M., Wellesley College, Wellesley, Mass.

SNYDER, PROF. CHARLES D., Johns Hopkins University Medical School, Baltimore, Md.

SOLLMAN, DR. TORALD, Western Reserve University, Cleveland, O.

SONNEBORN, DR. T. M., Johns Hopkins University, Baltimore, Md.

SPEIDEL, DR. CARL C., University of Virginia, University, Va.

STABLER, MR. ROBERT M., Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.

STARK, DR. MARY B., New York Homeopathic Medical College and Flower Hospital, New York City, N. Y.

STEWART, DR. DOROTHY R., Skidmore College, Saratoga Springs, N. Y.

STOCKARD, PROF. C. R., Cornell University Medical College, New York City, N. Y.

STOKEY, DR. ALMA G., Mount Holyoke College, South Hadley, Mass.

STRONG, PROF. O. S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.

STUNKARD, DR. HORACE W., New York University, University Heights, N. Y.

STURTEVANT, DR. ALFRED H., California Institute of Technology, Pasadena, Calif.

SUMWALT, DR. MARGARET, University of Pennsylvania Medical School, Philadelphia, Pa.

SWETT, DR. FRANCIS H., Duke University Medical School, Durham, N. C.

TAFT, DR. CHARLES H., JR., University of Texas Medical School, Galveston, Tex.

TASHIRO, DR. SHIRO, Medical College, University of Cincinnati, Cincinnati, O.

TAYLOR, WILLIAM R., University of Michigan, Ann Arbor, Mich.

TENNENT, PROF. D. H., Bryn Mawr College, Bryn Mawr, Pa.

THATCHER, MR. LLOYD E., Spring Hill, Tenn.

TRACY, PROF. HENRY C., University of Kansas, Lawrence, Kans.

TREADWELL, PROF. A. L., Vassar College, Poughkeepsie, N. Y.

TURNER, PROF. C. L., Northwestern University, Evanston, Ill.

TYLER, DR. ALBERT, California Institute of Technology, Pasadena, Calif.

UHLEMEYER, MISS BERTHA, Washington University, St. Louis, Mo.

UHLENHUTH, DR. EDUARD, University of Maryland, School of Medicine, Baltimore, Md.

UNGER, DR. W. BYERS, Dartmouth College, Hanover, N. H.

VAN DER HEYDE, DR. H. C., Galeria, Corse, France.

VISSCHER, DR. J. PAUL, Western Reserve University, Cleveland, O.

WAITE, PROF. F. C., Western Reserve University Medical School, Cleveland, O.

WALLACE, DR. LOUISE B., 523 Cowper Street, Palo Alto, Calif.

WARD, PROF. HENRY B., University of Illinois, Urbana, Ill.

WARREN, DR. HERBERT S., Department of Biology, Temple University, Philadelphia, Pa.

WARREN, PROF. HOWARD C., Princeton University, Princeton, N. J.

WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pa.

WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, N. D.

WHEELER, PROF. W. M., Zoölogical Laboratories, Harvard University, Cambridge, Mass.

WHERRY, DR. W. B., Cincinnati Hospital, Cincinnati, O.

WHITAKER, DR. DOUGLAS M., Stanford University, Stanford University, Calif.
WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pa.
WHITING, DR. PHINEAS W., University of Pittsburgh, Pittsburgh, Pa.
WHITNEY, DR. DAVID D., University of Nebraska, Lincoln, Nebr.
WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, O.
WILLIER, DR. B. H., The University of Chicago, Chicago, Ill.
WILSON, PROF. H. V., University of North Carolina, Chapel Hill, N. C.
WILSON, DR. J. W., Brown University, Providence, R. I.
WITSCHI, PROF. EMIL, University of Iowa, Iowa City, Ia.
WOGLOM, PROF. WILLIAM H., Columbia University, New York City, N. Y.
WOODRUFF, PROF. L. L., Yale University, New Haven, Conn.
WOODWARD, DR. ALVALYN E., Zoölogy Department, University of Michigan, Ann Arbor, Mich.
YOUNG, DR. B. P., Cornell University, Ithaca, N. Y.
YOUNG, DR. D. B., University of Maine, Orono, Me.
ZELENY, DR. CHARLES, University of Illinois, Urbana, Ill.

ISOLATION OF COLPIDIUM STRIATUM STOKES IN BACTERIA-FREE CULTURES AND THE RELATION OF GROWTH TO pH OF THE MEDIUM

ALFRED M. ELLIOTT

BIOLOGICAL LABORATORY, UNIVERSITY COLLEGE, NEW YORK UNIVERSITY

INTRODUCTION

Until rather recently, the many attempts to grow ciliates in bacteria-free cultures have met with little success. One of the first unquestioned cases is that of *Glaucoma piriformis*, which was isolated by Lwoff in 1922 and has been maintained in bacteria-free cultures for the past ten years (Lwoff, 1932). Peters (1921) had previously reported the establishment of bacteria-free cultures of *Colpidium colpoda*, but his results were criticised by Oehler (1924), who pointed out that the tests for bacteriological purity of the cultures were inadequate. Peters himself later reported (Gatenby and Cowdry, 1928, pp. 412–413) the recovery of bacteria from his cultures of *Colpidium*. Oehler, however, isolated strains of *Colpoda steinii* in 1916 and *C. cucullus* in 1923 (Oehler, 1924), and was able to maintain them on spinach broth. Milkovitch (1929) isolated another strain of *Glaucoma piriformis*, using a medium somewhat more complicated than that of Lwoff. In 1930 Glaser and Coria reported the establishment of bacteria-free strains of *Trichoda pura* and *Paramecium caudatum*, and more recently (Glaser, 1932) *Paramecium multimicronucleatum* has been added to the list. In addition, Hetherington (1932) has reported the establishment of bacteria-free cultures of *Colpidium campylum, Glaucoma scintillans* and *Loxocephalus sp.*

In the present investigation the writer has isolated *Colpidium striatum* in bacteria-free cultures and, as a necessary preliminary to further investigations, has attempted to devise satisfactory culture methods and to determine the relation of growth to pH of the medium. The writer wishes to express his appreciation to Professor R. P. Hall for suggestions during the course of the investigation.

MATERIAL AND METHODS

The bacteria-free strain of *Colpidium striatum* used in this investigation is a pure line derived from a single organism which was isolated from a hay infusion culture. By employing sterile micropipettes, de-

pression slides, Petri dishes and a 0.25 per cent tryptone medium a single organism was washed free of bacteria in the manner described by Parpart (1928) for *Paramecium*. It was found that when the bacteria were entirely eliminated, the *Colpidium* gradually decreased in size and then died after about two days. During the washing process, however, when a few bacteria were still present, the organism usually divided several times. These results finally led to the following procedure. A single ciliate was transferred to a tube of dextrose broth along with a few bacteria; abundant growth of the ciliates occurred. From this tube others were inoculated by the loop method, each loop containing approximately 10–20 ciliates together with a few bacteria. Growth of the ciliates was maintained through several series of such transfers, and by the plating method it was noted that the bacterial count was gradually decreasing. It was obvious at this point that the bacteria were not the sole source of food for the ciliates, since the concentration of protozoa was many times as great as that of the bacteria. This period of subculturing might be considered a sort of "acclimatization period" during which the number of bacteria is greatly reduced and the ciliates become more and more dependent upon nutritive substances in solution. After a number of series of such subinoculations, agar plates inoculated (1.0 cc.) from a number of culture tubes failed to show any bacterial colonies (duplicate series of plates were incubated at 37° C. and at room temperature for 10 days). In previous tests the presence of bacteria had been distinctly evident after 48 hours at 37° and after 4 days at room temperature. In the case of plates which showed no bacterial colonies, the colonies of ciliates were examined by the usual bacteriological staining methods as a confirmatory test. These bacteria-free ciliate colonies were then transplanted to liquid media, in which growth has since been maintained. Subsequent tests for bacteria, repeated at intervals over a period of several months, have been consistently negative.

Although a comparatively slow procedure, the method of isolation just described may have certain advantages. Most investigators have attempted to transfer ciliates directly from bacterized cultures to sterile media, and have failed to obtain growth in many cases; similarly, the writer's attempts to use this method were unsuccessful with *Colpidium* and several other ciliates. The more gradual elimination of bacteria by the dilution method was effective in the case of *Colpidium* and may prove to be so for other ciliates which cannot survive a sudden transfer from heavily bacterized to bacteria-free media.

In attempts to determine types of media satisfactory for growth of *Colpidium striatum* various kinds of Difco peptones and bacteriological media were tried. Difco dextrose broth gave excellent results, with

maximum populations of approximately 200,000 per cc. Inoculation of Durham fermentation tubes containing phenol-red dextrose broth was followed by acid fermentation in 24 hours, indicating that *Colpidium* utilizes dextrose readily. Difco nutrient gelatin and litmus milk were fairly satisfactory. In the gelatin tubes stratiform liquefaction was evident after 24 hours (room temperature), and the depth of the liquefied zone increased steadily throughout the period of observation (5 days). In litmus milk the casein was coagulated and the indicator reduced after about 48 hours, and complete peptonization had occurred in about two weeks. It is obvious, therefore, that *Colpidium striatum* produces a gelatinase and an enzyme capable of hydrolyzing casein. A similar gelatinase has previously been reported by Lwoff (1932) in *Glaucoma piriformis*. Of the various peptones tested, Difco tryptone proved to be very satisfactory, and has therefore been used in some of the experiments described below.

For experimental purposes the ciliates were grown in 16 × 150 mm. Pyrex tubes plugged with cotton. With the exception of Series I, experimental cultures were incubated at a temperature of 28° C. in a water bath, the cultures being inclined on a wire rack at an angle of 45 degrees, 18 inches below a battery of six 100-watt light bulbs. In tubing the media, 9.6 cc. of the appropriate medium at the desired pH were measured directly into the culture tube by means of a Schellbach burette graduated to 0.1 cc.; after sterilization in the autoclave, the amount of medium in each tube was approximately 9.0 cc. The pH of the medium was adjusted by means of a LaMotte roulette comparator, readings being subject to an error of ± 0.1.

In determining amounts of growth, initial and final counts were made with a Sedgwick-Rafter counting chamber and a Whipple micrometer.

Growth in Relation to pH of the Medium

Series I

In this preliminary series to determine the optimum pH and growth range for *Colpidium*, the following medium was used:

KNO_3	0.5	gram
KH_2PO_4	0.5	"
$MgSO_4$	0.25	"
NaCl	0.10	"
$NaC_2H_3O_2$	1.5	"
Difco tryptone	3.0	"
Distilled water	1.0	liter

The addition of sodium acetate to the medium was expected to increase the growth rate of *Colpidium*, since this substance has been shown (Mainx, 1928; Jahn, 1932; Lwoff, 1932; Loefer, MSS) to have such

an effect on certain flagellates; later findings, however, have indicated that the reverse is true for *Colpidium*. The pH of the different sets of tubes after inoculation was determined (Table I), and the series was grown at room temperature for 96 hours; half of the tubes in each set being counted at 48 hours and the remainder at 96 hours. The initial count was 234 organisms per cubic centimeter; the 48-hour and 96-hour counts are indicated in Fig. 1. Very little, if any, growth occurred in the tubes having an initial pH of 5.0, 5.5 and 9.1; and there was no growth below pH 5.0.

In determining the pH of the various sets of tubes after 48 and 96 hours of incubation, respectively, it was noted that distinct changes had been produced in the majority of instances (Table I). The tubes with an initial pH of 7.5 and below showed a decrease in hydrogen ion concentration; those of 8.0 and higher showed an increase.

The counts indicated that maximum growth had occurred in the tubes with an initial pH of 6.6; however, the pH of these tubes had risen to 7.3 at 48 hours and to 8.2 at 96 hours. It was apparent that the medium was not buffered sufficiently to maintain a reasonably constant pH during the period of incubation, and in later series the composition of the medium was changed in attempts to meet this difficulty.

Series II

The medium used in this series was made up with increased amounts of buffer substances in the hope of preventing some of the pH changes noted in Series I:

KNO_3	0.5	gram
KH_2PO_4	1.0	"
$MgSO_4$	0.25	"
NaCl	0.10	"
Difco tryptone	8.0	"
Distilled water	1.0	liter

With an initial count of 1630 per cc., the pH of the various sets of tubes (Table II) ranged from 3.5 to 9.7. The series was incubated for 96 hours in a water bath at a constant temperature of 28.0° C. Although changes in pH (Table II) were noted in the tubes of this series also, they were not quite so extensive as in Series I.

The growth range, in this series, extended from 4.2 to 8.6 (initial pH), although very little growth occurred in the tubes at the two ends of the range. A more interesting feature of this series is the fact that, at 48 hours, maximum growth (Fig. 2) had occurred in the tubes with an initial pH of 5.5; those at 6.9 ranked second; while the tubes at 6.2 and 6.4 showed distinctly less growth. At 96 hours the maximum growth had shifted to the tubes with an initial pH of 6.4, while those at 5.5

ranked second, with a distinctly lower count at 6.2. These unexpected results were complicated by pH changes in the medium during growth of the ciliates, but they suggest that two different pH zones are favorable to growth of *Colpidium*, a condition which was not apparent in the medium (containing sodium acetate) used in Series I.

TABLE I

Initial pH	pH at 48 hours	pH at 96 hours	Change in pH
3.5	3.8	3.9	0.4
4.2	4.3	4.3	0.1
4.6	4.7	4.7	0.1
5.0	5.4	5.4	0.4
5.5	5.5	5.5	0.0
6.2	6.9	7.8	1.6
6.6	7.3	8.2	1.6
7.2	7.7		
7.5	7.8	8.0	0.5
8.0	7.9	7.9	−0.1
8.4	7.9	7.9	−0.6
8.6	7.9		
9.1	8.5	8.5	−0.6

TABLE II

Initial pH	pH at 48 hours	pH at 96 hours	Change in pH
3.5	3.5	3.5	0.0
4.2	4.2	4.2	0.0
4.6	4.6	4.6	0.0
5.0	5.0	5.2	0.2
5.5	6.5	6.6	1.1
6.2	7.1	7.2	1.3
6.4	7.3	7.5	1.2
6.9	7.5	7.7	0.8
7.5	7.7	7.8	0.3
8.2	7.9	7.9	−0.3
8.4	8.2	8.2	−0.2
8.6	8.5	8.4	−0.2
9.7	9.7	9.7	0.0

Series III

In view of the differences between Series I and II it was decided to compare growth with and without sodium acetate, and also to add more tryptone to the medium in the hope of decreasing the pH changes. The medium was the same as that in Series II, except that 10 grams of tryp-

tone were used instead of 8. To half of the medium, sodium acetate was added in a concentration of 0.2 per cent, and the pH of different portions of each was adjusted to the desired points. The initial pH of each set

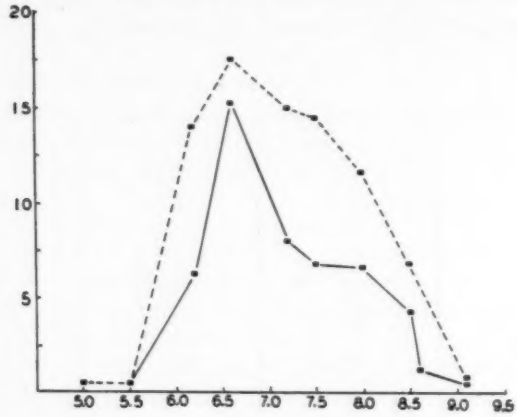

FIG. 1. Concentration of ciliates in thousands per cubic centimeter is plotted against initial pH. The solid line indicates growth at 48 hours; the broken line, growth at 96 hours.

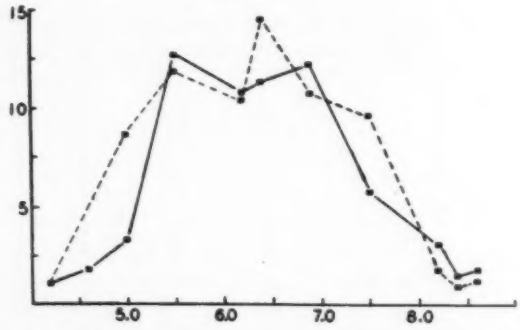

FIG. 2. Series II. Final concentration in thousands per cubic centimeter plotted against initial pH. The solid line indicates growth at 48 hours; the broken line, growth at 96 hours.

of tubes (Table III) was determined after inoculation. The initial count was 300 per cc. Counts were made (Fig. 3) after incubation for 96 hours in the water bath.

The final counts showed clearly that sodium acetate exerts a distinct effect on *Colpidium striatum* but that, contrary to expectations, the

TABLE III

With sodium acetate			Without sodium acetate		
Initial pH	pH at 96 hours	Change in pH	Initial pH	pH at 96 hours	Change in pH
3.5	3.5	0.0	3.5	3.5	0.0
4.0	4.0	0.0	3.8	3.8	0.0
4.5	4.5	0.0	4.5	4.5	0.0
5.2	5.2	0.0	5.0	5.0	0.0
5.5	5.5	0.0	5.5	5.5	0.0
6.2	6.2	0.0	5.7	5.8	0.1
6.4	6.4	0.0	6.5	7.0	0.5
7.0	7.5	0.5	7.0	7.5	0.5
7.8	7.9	0.1	7.6	7.6	0.0
8.2	8.2	0.0	8.0	8.0	0.0
8.6	8.6	0.0	8.2	8.2	0.0
8.8	8.8	0.0	8.6	8.7	0.1
9.5	9.5	0.0	9.5	9.5	0.0

growth rate is decreased rather than increased as in Euglenida and Phytomonadida. In addition, the pH range which permits growth is narrower than in the medium without sodium acetate: 6.2 to 7.8 in the

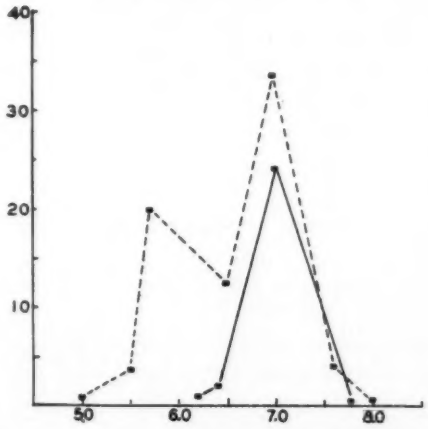

FIG. 3. Series III. Concentration of ciliates in thousands per cubic centimeter is plotted against initial pH. The solid line indicates concentration in the sodium-acetate medium; the broken line, the medium without sodium acetate.

acetate medium, and 5.0 to 8.0 in the medium without acetate. While maximum growth occurred in the tubes with an initial pH of 7.0 for each type of medium, the medium without acetate showed a second high point

at pH 5.7, a type of growth similar to that noted previously in Series II. As indicated in Table III, the pH changes were relatively slight in this more heavily buffered medium; hence, it seems obvious that there are two "optima" for growth of *Colpidium* in the medium containing no sodium acetate, whereas only a single optimum is noted in the acetate medium (see also Series I).

Series IV

At this point in the investigation it seemed desirable to determine whether the "bimaximal" type of growth could be observed in a different medium. Consequently, Difco dehydrated dextrose broth (5

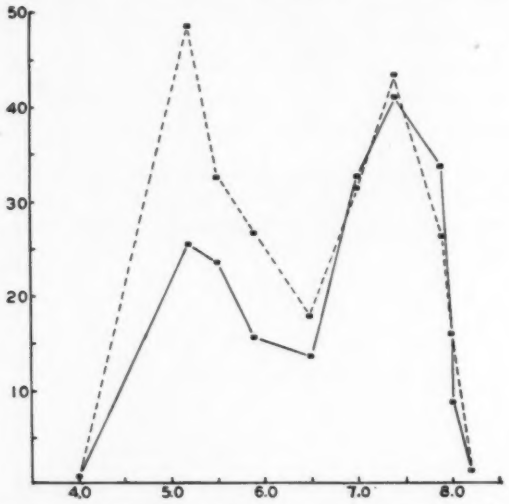

FIG. 4. Series IV. Concentration of organisms in thousands per cubic centimeter is plotted against initial pH. The solid line indicates concentration at 48 hours; the broken line, at 96 hours.

grams) was substituted for tryptone in the medium used in Series I. The initial count was 900 organisms per cubic centimeter. Preliminary counts were made at 48 hours, and final counts (Fig. 4) at 96 hours of incubation in the water bath.

At 48 hours maximum growth had occurred in the tubes with an initial pH of 7.4, with a second distinct high point at 5.2. At the end of 96 hours, growth in the 5.2 tubes had exceeded that at 7.4. In this case the bimaximal type of growth was even more evident than in the preceding series; allowing for observed changes in pH (Table IV), the lower "maximum" in this case lies between pH 5.2 and 5.5, and the upper between 7.4 and 7.6.

TABLE IV

Initial pH	pH at 48 hours	pH at 96 hours	Change in pH
4.0	4.0	4.0	0.0
5.2	5.4	5.5	0.3
5.5	6.5	6.8	1.3
5.9	6.7	7.0	1.1
6.5	7.0	7.2	0.8
7.0	7.2	7.5	0.5
7.4	7.5	7.6	0.2
7.9	7.6	7.7	−0.2
8.0	7.7	7.7	−0.3
8.2	7.8	7.8	−0.4
8.2	7.8	7.8	−0.4

BIMAXIMAL GROWTH IN RELATION TO FINAL pH

In relation to initial pH of the medium, the growth "maxima" (as indicated by final counts) were as follows: Series II, 5.5 and 6.4; Series III, 5.7 and 7.0; Series IV, 5.2 and 7.4. On comparing the final counts

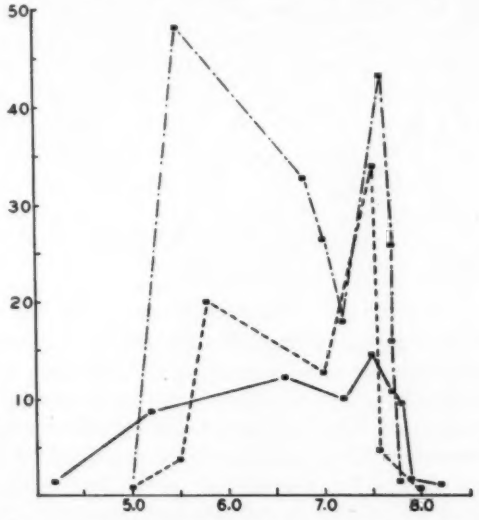

FIG. 5. Concentration of organisms in thousands per cubic centimeter is plotted against final pH in Series II (————), III (— — — — —) and IV (— - — - — -).

with the final pH, the maxima lie as follows (Fig. 5): Series II, 6.6 and 7.5; Series III, 5.8 and 7.5; Series IV, 5.5 and 7.6. In Series II, the initial pH 6.4 had shifted to 7.3 at 48 hours, so it seems obvious that in

the growth of *Colpidium striatum* in relation to pH, one optimum lies above 7.0 (perhaps near 7.5–7.6), while the other lies in the acid range, the lowest optimum (5.5) being observed in dextrose broth medium.

Division Rate in Relation to Final pH

Conflicting results have been reported concerning the relation of hydrogen-ion concentration to division rate in ciliates. Darby (1929) demonstrated, within the limits of his experiments, that *Paramecium caudatum* and *P. aurelia* show a marked difference in division rate in media of varying pH values. Phelps (1931), however, found that *P. aurelia,* grown on one strain of bacteria, showed no difference in division rate between pH limits of 5.9 and 7.7. He attributed any change in fission rate, within these limits, to a change in the available bacterial food and not to the direct action of pH. However, in comparing the average fission rate per day of *Colpidium striatum,* calculated from final counts using the formula,

$$n = \frac{\log b - \log B}{\log 2},$$

with final pH, the following differences were noted: In Series II, at pH 5.2 and 7.8 the fissions per day were 0.60 and 0.64 respectively; while at the optima, pH 6.6 and 7.5, the rates were 0.72 and 0.80. In Series III, at pH 5.0 and 7.6 the fission rates were 0.36 and 0.94; at the optima, pH 5.8 and 7.5, the rates were 1.52 and 1.71. Likewise in Series IV, at pH 5.5 and 7.7, the fission rates were 1.44 and 1.04 respectively; at the alkaline optimum (pH 7.6), 1.39 fissions per day. These results indicate that, within the range mentioned by Phelps, the division rate of *Colpidium striatum* varies with pH of the medium, as previously maintained by Darby (1929) for *Paramecium.*

Discussion

The literature on the relation of hydrogen-ion concentration to the growth of ciliates is not very extensive, and at the same time somewhat contradictory results have in certain instances been reported for the same species. A survey of the information available indicates that *Colpidium striatum* is tolerant to a somewhat wider range of hydrogen-ion concentration than is the case for a number of other ciliates. On the basis of the results described above, *C. striatum* will grow in media with initial pH of 4.0 to 8.6, with a narrower range in a medium containing sodium acetate. The range is somewhat broader than that (approximately 6.0 to 8.5) described by Pruthi (1926) for *Colpidium sp.,* but agrees fairly well with the findings of Mills (1931) that the rate of food-vacuole formation increases from pH 4.5 to 6.0 and then decreases to zero at pH 8.0. Other ciliates show a more restricted pH range. For example, the range for *Spirostomum ambiguum,* as reported by Saunders

(1924), is pH 6.8 to 7.8, and according to Morea (1927), pH 6.5–8.0. Darby (1929) stated that in *Paramecium caudatum* division occurs between pH 5.3 and 8.2; *P. aurelia,* according to the same author, grows at pH 5.7–7.8, while Morea (1927) stated that this species would live at pH 6.0 to 9.5. On the other hand, *P. multimicronucleatum* is more nearly comparable with *Colpidium striatum,* since it grows at pH 4.8–8.3 (Jones, 1930). An interesting feature in the growth of *Colpidium striatum* is the presence of two pH "optima." Loefer (1932) has noted a similar situation in the growth of *Chilomonas.* Hopkins (1928), in comparing the relation of pH to rate of locomotion in *Amœba proteus,* obtained a double optimum with an intermediate low point at pH 7. Likewise, Mast (1931), in comparing length of life with pH in the same form, observed one optimum at pH 6.6–7.0 and another at 5.0–6.4. In growth of *Colpidium striatum* one optimum appears above pH 7.0 and another below pH 7.0. An explanation of this bimaximal type of growth range in Colpidium is not yet available, but it may possibly involve the activities of different types of enzymes elaborated by the ciliates.

The effect of sodium acetate on *C. striatum* was surprising. Various investigators (Mainx, 1928; Jahn, 1932; Lwoff, 1932; Loefer, MSS) are agreed that this substance greatly accelerates the growth of certain flagellates. Such reports have been confirmed by the writer in the case of *Hæmatococcus pluvialis;* this flagellate was grown in the acetate medium used for *Colpidium* as a check on the results obtained with the ciliate—growth of *Hæmatococcus* was distinctly accelerated by sodium acetate. Collett (1919), however, observed that the acetate radical, among others, was toxic to both *Paramecium* and *Euplotes.* Mast (1931) also found that acetate in N/1000 concentration was more toxic than several other anions ($-SO_4$, $-NO_3$, $-Cl$, $-PO_4$). In the case of *C. striatum,* addition of sodium acetate to the medium brings about a definite depression of the division rate. At the same time, the growth range in relation to pH is distinctly narrowed, and the two pH "optima" (seen in media without acetate) are replaced by a single optimum. The significance of these peculiar effects of acetate on *Colpidium* is still unknown.

Summary

The method used in isolating *Colpidium striatum* in bacteria-free cultures is outlined, and suitable culture media are described. Growth of the ciliates was found to occur within the pH range 4.0 to 8.6. Within this range two growth "maxima" were observed, one above and one below pH 7.0. Addition of sodium acetate to the medium restricted the growth range, depressed the fission rate, and brought about

a replacement of the two growth "maxima" by a single maximum. Division rate was found to vary with the pH of the medium. Acid fermentation of dextrose, liquefaction of gelatin, and peptonization of litmus milk were observed.

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MARINE BACTERIA AND THEIR RÔLE IN THE CYCLE OF LIFE IN THE SEA

I. Decomposition of Marine Plant and Animal Residues by Bacteria [1]

SELMAN A. WAKSMAN, CORNELIA L. CAREY,[2] AND HERBERT W. REUSZER

(From the Woods Hole Oceanographic Institution and the New Jersey Agricultural Experiment Station)

The synthesis of organic matter in the sea through the agency of the chlorophyll-bearing plants, ranging from the smallest diatoms to the largest algæ, consists in the assimilation of the carbon as CO_2, of the nitrogen as nitrate, and probably to a lesser extent as ammonia and nitrite, of the phosphorus as phosphate, and of other elements in lower concentrations. Before these elements can be returned to circulation, after the plants as well as the animals which fed partly upon them die, the complex organic substances have to be decomposed through the agency of bacteria. If one is to judge by analogy with the decomposition processes which take place on land, the liberation of the elements in a mineralized form does not represent a simple process, but rather a chain of processes. The rate of liberation of the elements by bacterial action depends primarily upon the chemical composition of the materials undergoing decomposition and upon the organisms active in the decomposition processes.

The following investigations were undertaken for the purpose of determining to what extent the chemical composition of marine residues of plant and animal origin influences the rate of their decomposition by marine bacteria and the rate of liberation of the important elements, especially nitrogen, in an available form. It was further essential to obtain light upon the mechanism of decomposition of some of the chemical constituents of the marine residues by specific members of the bacterial population of the sea. These investigations can be classified, therefore, under three distinct headings, namely: (1) the chemical composition of marine zoöplankton and of certain marine algæ, (2) the decomposition of the plankton and algal material in sea water and in marine

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[2] Department of Botany, Barnard College, Columbia University.

mud by bacteria, (3) the decomposition of certain algal constituents by specific bacteria isolated from the sea.

Materials Used and their Chemical Composition

Two types of organic matter of marine origin were used in this investigation, one representing zoöplankton and one algal material. The zoöplankton was collected on July 6, 1932, in Buzzard's Bay, by means of an ordinary scrim plankton net. As soon as brought to the laboratory, the excess liquid was pressed out through cheesecloth and through a fine silk net, and plankton then dried in a current of warm air. The plankton consisted largely of copepods (*Centropages hamatus* and *C. typicus*), a few isopods, decapods, *Sagitta elegans*, fragments of hydroids, and fish eggs. The liquid pressed out by ordinary hand pressure from the original plankton was examined and found to consist largely of bacteria and some colloidal material.

The air-dry plankton contained 6.7 per cent moisture. This material was subjected to analysis and the results calculated on a dry basis. The total dry plankton was found to consist of 7.88 per cent nitrogen, 28.1 per cent ash, of which 15.1 per cent was sea salt as calculated from the chlorine content. The complete proximate analysis (22) of the plankton is given in Table I.

Table I

Proximate analysis of zoöplankton. Dry, salt-free basis.

Chemical constituent	Percentage
Ether-soluble fraction *	10.1
Alcohol-soluble, non-nitrogenous *	4.8
Cold water-soluble *	3.1
Hot water-soluble *	2.0
Chitin	5.8
Protein	55.7
Cellulose	2.4
Lignin (?)	2.8
Ash, salt-free	13.0
Total accounted for	99.7

* Ash and nitrogen-free basis.

The chitin content was determined on a separate sample, by repeated treatment with hot dilute hydrochloric acid (2 per cent) and hot 12 per cent KOH solution, then washing with alcohol and ether. The nitrogen of the chitin was subtracted from the total nitrogen of the material and the rest calculated as protein, using the factor 6.25. The cellulose content was calculated from the amount of sugar produced on hydrolysis of the plankton residue (after extraction with hot dilute acid) with 80 per cent sulfuric acid in the cold, diluting with 15 volumes of water and heating in order to complete the hydrolysis. The total sugar formed on

hydrolysis was corrected for the reducing sugar produced from the chitin and calculated as cellulose, using the factor 0.9. The cold water extract of the plankton was free from reducing sugar, while the hot water and the hot dilute acid extracts were free from glycogen and hemicellulose. Just as in the case of the alcohol extract, the cold and hot water-soluble fractions were corrected for ash and nitrogenous constituents.

The danger of using alcohol for the preservation of the plankton is brought out by the fact that when treated with 95 per cent ethyl alcohol, the total plankton gave 24.7 per cent alcohol-soluble material, of which 23.2 per cent was organic matter. The low figure reported in the table for the alcohol-soluble fraction is due to the fact that in the complete analysis the dry plankton was first extracted with ether, while the total alcohol-soluble portion was corrected for ash and protein, so as to avoid duplicating the same constituent in the final analysis; since a large part of the ether-soluble material and some of the nitrogenous constituents are also soluble in alcohol, the corrections made resulted in reducing the concentration of the remaining alcohol-soluble fraction.

The results of this analysis are quite in accord with similar analyses made by Brandt (2), who employed, however, the methods commonly used in foodstuff analysis, whereby the chemical constituents were reported as crude fat, protein, carbohydrate, crude fiber, and nitrogen-free extractives.

It is important to call attention, in connection with this analysis, to the high content of organic nitrogenous constituents in the zoöplankton as well as of fatty substances and of chitin, and to the low content of carbohydrates. The amount reported as cellulose is so small that one may be justified in questioning whether this represents true cellulose or other complexes, which give, in addition to chitin, reducing substances on hydrolysis with concentrated acid; the presence of a small amount of cellulose may also be due to certain constituents of plant origin. The same is true of the lignin; the concentration of this complex is so small that its origin in the plankton may still be considered as an open question; it may actually represent a certain polyuronide not readily hydrolyzed by 80 per cent sulfuric acid in the cold (14).

As sources of algal material, *Fucus vesiculosus, F. platycarpus,* and *Ulva lactuca* were selected. In the case of the *Fucus,* the material was brought to the laboratory as soon as collected, cleaned thoroughly by hand to remove extraneous matter, washed 2–3 times with tap water, then with distilled water to remove the excess of salt, and dried at about 80–90° C. In the case of the *Ulva,* however, the material was dried directly, without preliminary washing. The results of the analysis of these three algal materials are reported in Table II.

The cold water-soluble fraction of the *Fucus* contained 1 to 2.5 per cent reducing sugar, on the basis of the total dry material. The hot water-soluble fraction, when hydrolyzed with dilute acid, gave 2.8 to 3.5 per cent reducing sugar, pointing to the presence in this fraction of complex carbohydrates. After the hot water extraction, the material was treated with 2 per cent hydrochloric acid, at 100° C., for 5 hours; the extract was removed by filtration, neutralized and analysed for reducing sugar; the results obtained are reported as "hemicelluloses." However,

TABLE II

Chemical composition of three marine algæ. Dry, salt-free basis.

Organism	*Fucus vesiculosus*	*F. platycarpus*	*Ulva lactuca*
Chemical constituent:	*per cent*	*per cent*	*per cent*
Ether-soluble fraction	2.3	2.3	0.8
Alcohol-soluble fraction	6.7	8.7	10.6
Cold water-soluble organic matter	13.6	12.1	17.9
Hot water-soluble organic matter	7.8	8.1	10.7
"Hemicelluloses," as reducing sugars	11.2	11.8	11.0
Uronic acid anhydride	27.5	16.1	6.2
"Pentosan," by furfuraldehyde method	28.7	24.5	31.4
"Cellulose," as reducing sugar	4.3	5.4	6.2
"Lignin," or complexes not acted upon by 80 per cent H_2SO_4 in the cold *	5.6	6.2	2.9
Protein	5.4	4.7	12.4
Ash	13.9	14.6	13.6

* This preparation not acted upon by cold 80 per cent H_2SO_4 and boiling 5 per cent H_2SO_4 is not lignin, but a largely carbohydrate-like complex. It is of interest to note that Butler (4) reported the presence of 5.21 per cent "crude fiber" in *F. vesiculosus* and that Schmidt (23) found that 40 per cent of the polyglycuronic acid in *F. serratus* was insoluble in cold 80 per cent H_2SO_4.

this figure does not represent the total hemicellulose, including both true polysaccharides and polyuronides; when separate samples were analyzed for uronic acid and for total pentosan (furfuraldehyde method), considerably larger figures were obtained than those given under "hemicelluloses." This is due to the fact that the furfuraldehyde formed on treatment of the material with 12 per cent hydrochloric acid may be due to substances consisting not only of pentoses derived from pentosans but also of uronic acid complexes forming polyuronides. The latter change on hydrolysis only incompletely to reducing sugar. The three sets of results for the carbohydrate groups, namely the reducing sugar formed on hydrolysis with dilute acid, the pentosan, and the uronic acid anhydride, overlap and render it, therefore, impossible to make a final addi-

tion of the results. The fractions reported as "cellulose" and "lignin" do not necessarily represent the corresponding complexes in higher plants, for considerations similar to those presented above for these constituents in the zoöplankton.

The results of the analyses of the algal materials are also in line with those found by others. These algæ are usually reported (3) to contain from 1.37 to 3.71 per cent nitrogen, 1.50 to 5.21 per cent crude fiber, pointing to a low cellulose and lignin content, and 15.7 to 33.2 per cent ash. The organic complexes are made up largely of carbohydrates, which could be classified with the hemicelluloses, including both true saccharides and polyuronides (8). Although a few specific carbohydrates have been definitely demonstrated (8, 10) in algal material, such as mannitol, arabinose, galactose and methyl pentose or fucose, most of the constituents are still imperfectly described under such names as algin, laminarin, fucoidin, fucin, etc. Some of these complexes represent polyuronides, as in the case of the polymer of mannuronic acid, which is readily isolated from *Fucus* by dilute alkali solutions (1, 11).

Decomposition of Zoöplankton by Marine Bacteria

Our knowledge of the function of bacteria in decomposing the plant and animal residues in the sea is still very limited, the available information being largely based upon speculation rather than upon experimental evidence. It was early recognized that the bacteria active in the decomposition processes in the sea also synthesize new organic complexes in the form of bacterial cell substance. Vernon (17), for example, reported that the largest amount of purification of ocean waters is carried on through bacterial agency; when sea water was kept in the darkness the bacteria removed a large part of the ammonia in the water and synthesized considerable slime; larvæ grown in this water were larger than normal, which led Vernon to conclude that the bacterial cells serve as nutrients for these larvæ. The energy necessary for this bacterial synthesis is no doubt derived from the dissolved organic substances in the water, which have been found (12) to range from 3 mg. of carbon per liter of water in the Baltic Sea to 11–14 mg. in the Kiel Fjord. However, Moore (9) concluded that only a maximum of 1 mg. of organic matter is dissolved in one liter of water. According to Gran and Ruud (5) and Krogh (7), the organic matter in solution is greatly in excess over that present as plankton; this organic matter was looked upon as mainly a waste product, which is not readily available for animal nutrition, and only to a limited extent to bacterial development. Korinek (6) has shown that when fresh water bacteria are placed in the sea they are unable to attack algal residues, while marine bacteria are able to bring

about active decomposition of the algal material; however, no quantitative measurements were made. The decomposition of marine algæ added to the soil brought out the fact (15) that the larger the nitrogen content of the algæ the greater is the amount liberated as ammonia during decomposition.

In the following investigations on the decomposition of marine plant and animal residues by bacteria, not only was there an attempt made to study the rate of decomposition and the liberation of nitrogen as ammonia, but also to establish definite relationships between the composition of the substrate and the rate and nature of its decomposition.

For the study of the decomposition of the zoöplankton, 150-cc. portions of fresh sea water were placed in a series of 300-cc. long-necked flasks; another series of flasks received 100-gram portions of fresh marine mud (containing about 50 per cent moisture) and an additional

TABLE III

Decomposition of zoöplankton by bacteria in sea water and in marine mud *

Medium used	Amount of plankton added	CO_2 liberated		NH_3 liberated
		total	above control	
		mgm. C.	*mgm. C.*	*mgm. N.*
Water.....	0	5.6	—	0.10
Mud.....	0	3.2	—	0.25
Water.....	1 gram	69.7	64.1	40.30
Mud.....	1 gram	103.9	100.7	38.50

* Period of decomposition—19 days.

50 cc. of fresh sea water. Some of the flasks were left as controls, while some received one-gram portions of the air-dry plankton. All the flasks were connected, in the laboratory (16–20° C.), with an aëration apparatus (21), and aërated daily for about one hour. The air, first freed from CO_2, was made to bubble through the liquid of the culture or through the surface layer of water, in the case of the mud cultures, and was then passed through tubes containing standard $Ba(OH)_2$ solution, where the CO_2 liberated in the process of decomposition was absorbed. The experiment was allowed to proceed for 19 days.

At the end of the decomposition period, 2-cc. portions of concentrated H_2SO_4 were added to each flask and the cultures thoroughly aërated for about 1 to 2 hours, so as to liberate the CO_2 absorbed in the water. The flasks were then disconnected and analyzed for ammonia, by distillation with MgO; in the case of the mud cultures, the material

was first extracted with N/1 KCl solution, and the ammonia determined in the extract. The cultures contained no nitrite or nitrate. The results of the decomposition of the zoöplankton in the sea water and in the mud are given in Table III, while the course of evolution of CO_2 is illustrated graphically in Figs. 1 and 2. The precipitous rise in the amount of CO_2

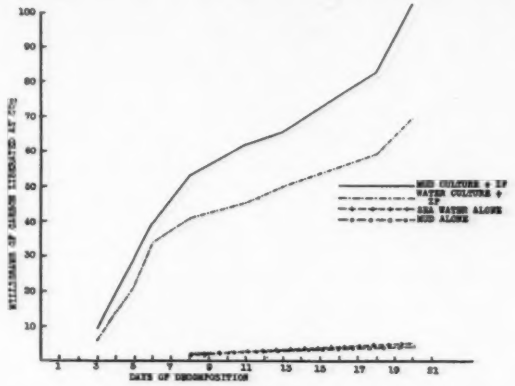

FIG. 1. Accumulated evolution of CO_2 in the course of decomposition of zoöplankton in sea water and in marine mud cultures.

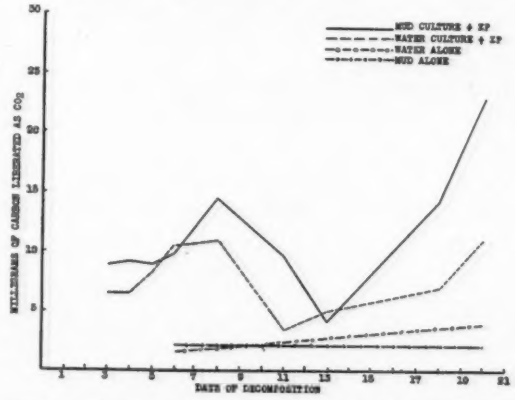

FIG. 2. Daily evolution of CO_2 in the decomposition of zoöplankton in sea water and in marine mud cultures.

at the end of the decomposition period is due to the liberation by the final acid treatment of the CO_2 present in the medium as carbonate and bicarbonate.

The amount of CO_2 liberated from the plankton in the mud as a

medium is much greater than in the sea water, while the amount of nitrogen liberated as ammonia is practically the same in both media. Since one gram of the air-dry plankton contained 625 milligrams of organic matter (allowing for moisture and total ash), with about 300 milligrams of total carbon, the liberation in the mud medium of 100 milligrams of carbon as CO_2 within 19 days indicates a very rapid decomposition of the plankton. However, more than a half of the total nitrogen in the plankton has been liberated as ammonia in the same culture and during the same period of decomposition; this points to two definite alternatives: 1. The various chemical constituents of the plankton are not all decomposed at the same rate. 2. More of the plankton is actually decomposed than one is able to measure by the CO_2 evolution, since considerable synthesis of bacterial cell substance takes place in the process of decomposition. The first suggestion is borne out by an examination of the curves in Fig. 2. Although the rate of decomposition had come to a minimum in 11 days, in the case of the sea water medium,

TABLE IV

Decomposition of marine algæ by bacteria in sea water and in marine mud *

Medium used	*Ulva lactuca*			*Fucus vesiculosus*		
	CO_2 liberated		NH_3–N liberated	CO_2 liberated		NH_3–N liberated
	total	above control †		total	above control †	
	mgm. C.	*mgm. C.*	*mgm. N.*	*mgm. C.*	*mgm. C.*	*mgm. N.*
Water	89.0	83.4	5.2	16.0	10.4	0.4
Mud	105.0	101.8	4.1	89.0	85.8	—

* 1 gram of air-dry material used; 26 days incubation for *Ulva* and 28 days for *Fucus*.

† Water and mud controls were the same as in Table III.

and in 13 days, in the case of the mud medium, further decomposition began to take place after that date.

These results suggest the following possibility: some of the constituents of the plankton, probably the proteins, are undergoing active decomposition during an early stage; this is later followed by the decomposition of other constituents of the plankton, probably the chitins and the fatty substances. The specific bacteria capable of attacking these two complexes are probably more active in the mud than in the water. Their decomposition of these substances which are low in nitrogen or are totally free from nitrogen will not only not result in any ammonia liberation but may actually result in ammonia consumption by bacteria for cell

synthesis. These two considerations may fully account for the greater decomposition of the plankton in the mud than in the water, as measured by the CO_2 evolution, not accompanied, however, by greater ammonia liberation.

Decomposition of Marine Algæ by Bacteria

The decomposition of the algal material by bacteria was carried out in a manner similar to that of the plankton. The period of decomposition was 26 days for the *Ulva* and 28 days for the *Fucus*. One-gram quantities of the air-dry material of *F. vesiculosus* and *U. lactuca* were added to the flasks containing the water as a medium or the mud and water; the cultures were aërated daily.

The results presented in Table IV and Fig. 3 show that the *Ulva* was decomposed by the bacteria in the water and in the mud more actively than the *Fucus*. In the case of the *Ulva* there was very little difference in the rate of decomposition in the water or in the mud; the

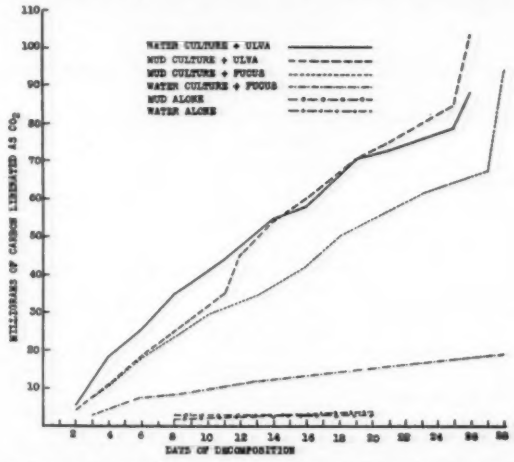

Fig. 3. Course of evolution of CO_2 in the decomposition of marine algæ in sea water and in marine mud.

Fucus, however, showed a very marked difference, since a considerably greater amount of CO_2 was liberated in the mud than in the water cultures. About 4 to 5 milligrams of ammonia-nitrogen were accumulated in the decomposition of the *Ulva*, while there was no ammonia at all or only a mere trace in the *Fucus* cultures. The nitrogen content of the two algæ offers the explanation for this difference. *Ulva* contained 2.56 per cent nitrogen on a total dry, ash and salt-free basis; *Fucus*, however, contained only 1.01 per cent nitrogen, on an ash and salt-free basis.

The difference in the nitrogen content of the two materials has an important effect upon the rapidity and nature of their decomposition. It has been found (23) in the decomposition of plant residues in soils and in composts that if the material contains 1.7 per cent nitrogen, the latter is just sufficient for the active decomposition of the plant substance, without any nitrogen being liberated as ammonia and without any added nitrogen required by the microörganisms. When the plant material contains more than 1.7 per cent nitrogen, the excess will be liberated as ammonia. However, when the nitrogen content is less than 1.7 per cent, some inorganic or available form of nitrogen must be added to enable the bacteria and the fungi to decompose the plant material.

The same law seems to hold true also for the decomposition of algal residues in the sea. There was more than enough nitrogen in the *Ulva* to bring about its rapid decomposition by the bacteria, as shown by the fact that out of 1 gram of the air-dry material, containing 590 milligrams of organic matter with about 265 milligrams carbon, there was liberated

TABLE V

Influence of addition of available nitrogen upon the decomposition of Fucus vesiculosus in sea water *

Treatment	CO_2 liberation		Inorganic nitrogen left	Inorganic nitrogen disappeared	Numbers of bacteria in 1 cc. of culture
	total	due to added nitrogen			
	mgm. C.	*mgm. C.*	*mgm. N.*	*mgm. N.*	
2 grams Fucus alone	18.9	—	0	0	14,400,000
2 grams Fucus + 50 mgm. $NaNO_3$	69.5	50.6	0	8.0	70,600,000
2 grams Fucus + 300 mgm. $NaNO_3$	54.7	35.8	0.5	47.5	31,000,000
2 grams Fucus + 50 mgm. $(NH_4)_2SO_4$	47.2	28.3	4.6	5.9	28,600,000

* 2-gram portions of air-dry *Fucus* in 150 cc. of water; decomposition, 28 days.

nearly 100 milligrams carbon as CO_2 or about 40 per cent of the total, both in the water and in the mud cultures. The *Fucus*, however, containing 827 milligrams of organic matter and about 372 milligrams of carbon in 1 gram of the air-dry material, did not contain sufficient nitrogen for its rapid decomposition by the bacteria.

The greater decomposition of the *Fucus* in the mud than in the sea water may be due to two factors: 1. The organic matter in the mud undergoes some decomposition and the amount of nitrogen liberated in this process may be sufficient for the need of the bacteria, thus enabling

them to bring about considerably greater decomposition of the *Fucus*. 2. There is also a possibility that the mud may have a greater abundance of specific bacteria capable of attacking certain specific carbohydrates in the *Fucus*, while the water may be lacking in such bacteria.

In order to study further the factors that are concerned in the decomposition of the nitrogen-poor *Fucus* by marine bacteria, the influence of available nitrogen, both as ammonia and nitrate, has been determined. A series of cultures were prepared using sea water as the medium. All the cultures received 2-gram portions of air-dry, ground material of *Fucus vesiculosus*. The course of decomposition was followed by measuring the evolution of CO_2, in a manner similar to that outlined above. At the end of the incubation period (28 days), the residual nitrogen left as nitrate or as ammonia was determined. The results of this experiment are given in Table V and in Fig. 4.

When no available nitrogen was added to the medium, only a limited amount of *Fucus* underwent decomposition. The addition of a small

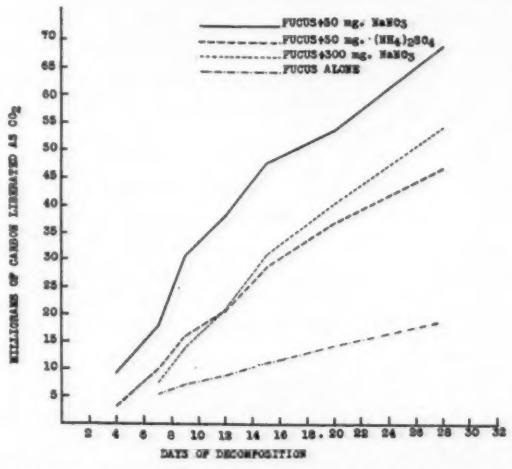

FIG. 4. Influence of available nitrogen upon the evolution of CO_2 in the decomposition of *Fucus vesiculosus* in sea water culture.

amount of nitrogen, in the form of an inorganic salt, brought about considerably greater decomposition, the nitrate nitrogen having a more favorable effect than the ammonium salt. An excess of nitrate, however, was less effective than a lower concentration. Where the lower amount of nitrate was added to the cultures, all the nitrogen was consumed by the bacteria bringing about the decomposition of the *Fucus*,

for the synthesis of bacterial cell substance. The cultures receiving an excess of nitrate showed a marked loss of the nitrate, through the process of denitrification. The fact that the nitrogen added to the cultures was required by the bacteria for the synthesis of cell substance can be further illustrated by the following considerations: in the decomposition of the algal material, the ratio between the carbon liberated as CO_2 and the nitrogen consumed by the bacteria, for the cultures receiving ammonium salt as a source of nitrogen, was 47.2 :5.9 = 8 :1; the corresponding ratio for the cultures receiving the lower nitrate concentration was 8.7 : 1. As will be shown later, these ratios are quite normal for the decomposition of carbohydrates by marine bacteria. There was no nitrate left in the cultures receiving the lower amount of nitrate, at the end of the decomposition period, and only a trace of nitrite was present; in the case of the higher nitrate concentration, only a small amount of nitrate was left at the end of the decomposition period, while a considerable amount of nitrite could be demonstrated in the medium.

The above results point definitely to the conclusion that nitrogen in the form of ammonia and nitrate can be used by marine bacteria for synthetic purposes, in the process of decomposition of nitrogen-poor algal material. When a high nitrate concentration is present, the *Fucus* material may be used as a source of energy by denitrifying bacteria for the reduction of the nitrate.

In order to determine what rôle the bacteria play in the decomposition of the *Fucus* material, 1-cc. portions of the various cultures were plated out, at the end of the decomposition period, using glucose-peptone sea water agar. The plates were incubated and the bacterial colonies counted at the end of 4 days. The results (Table V) show that there is a direct parallelism between the development of the bacteria and the liberation of CO_2, in the decomposition of *Fucus* material in sea water medium.

The results of these experiments prove conclusively that the rate of decomposition of marine algæ and the liberation of nitrogen as ammonia depend upon the chemical composition of the algal material. The more rapid decomposition of the *Ulva* than of the *Fucus* is due, aside from any difference in the chemical composition of the two, to the greater nitrogen content of the former. The more limited decomposition of the *Fucus* in the water cultures was due to a lack of available nitrogen, which prevented the development of the bacteria capable of decomposing the algal residues.

On comparing the results of Tables IV and V, one notes that there was a greater decomposition of the *Fucus* in the mud than in the sea water, even with the addition of an available source of nitrogen. This

suggests the possibility that nitrogen is only one factor limiting the decomposition of marine algal material in the sea. Possibly the mud contained other elements which are also needed for bacterial growth and which were not present in sufficient amounts in the sea water. The greater decomposition of the *Fucus* material in the mud may also be due to the presence in the latter of specific bacteria, which are able to decompose certain organic constituents of the *Fucus*, while these bacteria are not present in the free water.

Isolation of Cellulose and Hemicellulose-decomposing Bacteria from the Sea

In order to study further the mechanism of the decomposition of algal residues in the sea by bacteria, it was essential to isolate pure cultures of such bacteria and study their action upon certain purified algal constituents. The available information concerning the decomposition of specific constituents of marine plants by bacteria is largely limited to the agar-liquefying bacteria. Beginning with the work of Gran (4) in 1902, there were isolated from the sea a number of organisms capable of liquefying and decomposing agar. In most cases, as in the original form described by Gran, the agar was merely softened and, since no sugar was demonstrated, it is doubtful whether actual decomposition of the agar took place. Other organisms, as the form isolated from the marine mud of the Bahama banks and studied by Waksman and Bavendamm (18), decomposed the agar actively, liberating a large part of the carbon as CO_2; the bacteria, using the agar as a source of energy, were able to synthesize considerable cell substance.

Little is known concerning the occurrence in the sea of organisms capable of decomposing cellulose. Issatchenko (quoted in ref. 13) isolated from the mud of the salt lake Ssaky in Crimea two organisms, one of which decomposed cellulose without forming a pigment (*Bact. cellulosæ album*), while the other colored the paper yellow (*Bact. cellulosæ flavum*). Rubentschik (13) isolated from the salt limans in southern Russia an *Actinomyces* (*A. melanogenes*) capable of slow decomposition of paper. In 1929, Van der Lek (16) demonstrated the occurrence in the sea of *Bact. agar-liquefaciens*, which is capable of attacking cellulose and agar.

The following investigations deal with the occurrence in the sea of bacteria capable of attacking the various chemical constituents of marine residues as well as with the mechanism of the decomposition processes. The carbohydrate constituents were at first selected, due to their predominance in the algal residues. Three methods were employed for the isolation of the bacteria from sea water and from marine bottom material:

1. *The Silica-gel Method.*—Silica gel plates prepared by a method described elsewhere (19) are placed in flowing tap water, so as to bring about the removal of the excess of chlorides by dialysis; the dishes are then transferred to boiled sea water and kept there for about 1 hour; the last step may be repeated, using another portion of freshly boiled sea water, to insure sterility. The dishes are carefully removed from the water, the excess water drained off, and the surface covered with a small amount of powdered $CaCO_3$; for the purpose of isolating cellulose-decomposing bacteria, ground filter paper is placed on the surface of the gel; for the isolation of agar-decomposing organisms, powdered agar is used. A sterile salt solution consisting of 4 grams $NaNO_3$ and 2 grams K_2HPO_4 in 100 cc. of distilled water is then added to the surface of the medium, using 1-cc. portions for the small plates (15 cm. diameter) or 5-cc. portions for the large plates (25 cm. diameter). Plates so prepared were inoculated with fresh sea water, with marine bottom material or with pieces of algal growth.

2. *Enrichment Culture Method.*—This consists in placing 250-cc. portions of fresh sea water, 0.5 gram $NaNO_3$ and 0.1 gram K_2HPO_4, with or without 2-gram portions of $CaCO_3$, into a series of 250-cc. Erlenmeyer flasks. One per cent of cellulose, in the form of ground filter paper, or 1 per cent of dry agar were used as sources of carbohydrate.

3. *The Nutrient Agar Plate.*—This is made up to contain 1 gram of glucose, 1 gram peptone, 0.5 gram K_2HPO_4 and 15 grams agar in 1000 cc. of sea water. Marine mud, plankton tow, or fresh sea water are plated out on this medium, using various dilutions.

The cellulose-decomposing bacteria can be demonstrated most readily by the silica-gel plate method, while the agar-liquefying bacteria are best studied by the nutrient-agar plate method. In the case of the sea water, where only few bacteria are present, the enrichment culture may have to be used first, in order to demonstrate the presence of the specific organisms in the water. The cellulose bacteria developing on the silica gel plates are transferred to test tubes containing 5-cc. portions of sterile liquid medium (2 grams $NaNO_3$, 0.5 gram K_2HPO_4, 12 grams $CaCO_3$ in 1000 cc. sea water), with a strip of paper partly immersed in the liquid and partly above it. The agar-liquefying bacteria can be readily obtained in pure culture by the use of the agar plate; the colonies are picked and transferred to agar slants, and, if necessary, the process may be repeated using various dilutions of the culture. In the case of the cellulose bacteria, however, one must have recourse to the use of very high dilutions and repeated transfers in order to obtain pure cultures; this is true, of course, of the highly specific bacteria, which use cellulose as the only source of energy. When an organism is able to attack both

cellulose and agar, its purification does not represent any particular difficulties.

The presence in sea water and in the sea bottom of bacteria capable of attacking cellulose could easily be established. These organisms were found to be particularly abundant in the plankton tow. Attention has already been called (20) to the fact that a definite parallelism was found to exist between the numbers of bacteria and diatoms in the sea water and in the plankton; the suggestion has been made that bacteria live in the free water only to a very limited extent, but their life and activities in the sea are closely associated with the development of the plankton organisms, in addition, of course, to the bacteria inhabiting the sea bottom.

A sample of diatom tow obtained from George's Bank (Station 1335) (20) gave an abundant growth of cellulose-decomposing bacteria. Similar results were obtained when the water and bottom mud from several stations in the Gulf of Maine and George's Bank were used for the inoculation of silica gel plates. It was thus possible to demonstrate the presence in the sea of a number of cellulose-decomposing bacteria, which represent a variety of types, as determined by their morphological, cultural, and biochemical characteristics.

Morphologically, the marine cellulose-decomposing bacteria were found to comprise short rods, spiral-forming organisms, vibrios, and undulating elongated rods with tapering ends, characteristic of the Cytophaga group. One is able to demonstrate without any difficulty the presence in the sea of the three groups of cellulose-decomposing bacteria which have been classified by Winogradsky (24), namely, *Cytophaga* (elongated, flexuous rods), *Cellvibrio* (bent rods with rounded ends), and *Cellfalcicula* (spindle and sickle-shaped). In crude culture, the cellulose-decomposing bacteria were invariably accompanied by numerous protozoa, including flagellates, ciliates and amœbæ, which feed upon the bacteria. All the bacteria were actively motile.

The marine cellulose-decomposing bacteria produce in culture a variety of pigments, namely, pink, orange, yellow, brownish-yellow, or no pigment at all. Most of them are aërobic. Some anaërobic forms were also demonstrated, which were found capable of producing gas actively with cellulose as the only source of energy. These organisms were not isolated, however, in pure culture and were only insufficiently studied. As to the utilization of carbohydrates, some of the cellulose-decomposing bacteria were found to be highly specific, being unable to use any other source of energy, except cellulose. Some attacked not only cellulose but also agar and other hemicelluloses, as will be shown later. Some were able to utilize a variety of sugars.

Agar-liquefying bacteria are even more commonly found in the sea; they are especially abundant in the diatom tow and on marine algæ and their residues, where they seem to find the necessary nutrients for their development. It was pointed out elsewhere that 1 cc. of diatom tow was found to contain 2,100 to 2,500 cells of bacteria capable of liquefying agar; they made up 5.7 to 6.7 per cent of the total number of colonies developing on the plate.

A number of bacteria were isolated, either directly from the plate or after preliminary enrichment culture with cellulose or agar in sea water. Crude cultures of cellulose-decomposing bacteria on the silica gel plate or in enrichment cultures of cellulose in sea water invariably yielded agar-liquefying bacteria. In some cases, these bacteria could decompose both cellulose and agar, while in other cases the agar-liquefying organisms merely accompanied the cellulose-decomposing forms. The bacteria thus isolated were found to belong to several groups varying considerably in their physiological characteristics. No attempt will be made to describe and classify these organisms. Three cultures were selected for a more detailed study of the decomposition of carbohydrates; the cultural and morphological characteristics of only these cultures are presented here.

Culture No. 2 (b) was isolated from the silica gel plate containing cellulose as the only source of energy and $NaNO_3$ as a source of nitrogen. In crude culture it attacked both cellulose and agar and produced gas as a result of the decomposition of the agar. When freshly isolated, it produced lilac colored to pinkish-brown colonies, on the surface of the agar.

Culture No. 3 was also isolated from the same plate as No. 2 (b). It was found to form, however, two types of colonies, one pinkish in color and one colorless. The pinkish culture was later found to be the same as No. 2 (b), and was designated as 2 (a); it also decomposed both cellulose and agar. The second culture could not utilize cellulose, but acted upon agar alone and was designated as No. 3.

Culture No. 15 formed a yellowish to dirty-brown color upon agar and in liquid culture. It did not attack cellulose, but could utilize agar as the exclusive source of energy.

The agar used for these studies was the Bacto-Difco agar. For later studies, specially washed agar was prepared in quantity by the Digestive Ferments Co.

A brief description of these three agar-liquefying bacteria is given here.

Bacterium sp. No. 2. Forms long, slender rods; often filamentous and curved in young cultures. A few motile rods are present. Oc-

casionally there appear in these cultures a few extremely motile individuals, small in size, which seem to be identical with some of those found in Cultures 3 and 15. The organism reduces nitrate, decomposes cellulose, and does not liquefy gelatin.

Bacterium sp. No. 3. Forms very short rods, often coccus-like, with an equal proportion of slightly longer, slender rods scattered throughout the field. A few rods of the same length but much thicker appear with polar bodies. A few actively motile rods, often granular, sometimes having the appearance of two very short rods joined together, are also found. The latter appear more frequently among the non-motile forms. This organism liquefies gelatin, does not decompose cellulose, and does not reduce nitrate.

Bacterium sp. No. 15. Microscopically similar to No. 3, except that it contains more motile individuals and fewer coccus-like forms. Cultural characteristics are the same as those of No. 3.

Decomposition of Various Carbohydrates by Cellulose-decomposing and Agar-liquefying Bacteria

When agar or other constituents of marine algæ are decomposed by agar-liquefying bacteria, the carbohydrates are used by the bacteria chiefly as sources of energy, as well as for synthesis of bacterial cell

Table VI

Decomposition of agar by three marine bacteria. Milligrams of dry material.

Culture	Agar left*	Agar decomposed	CO_2 evolved as carbon	Agar left, as sugar†	Agar utilized	NO_3—N left	NO_3—N consumed	Ratio of carbon liberated as CO_2 to nitrogen consumed
Control	498	0	0	245	0	26.4	—	
Bacterium sp. No. 2	167	331	91.6	82	163	14.0	12.4	7.4
Bacterium sp. No. 3	144	354	74.7	71	174	13.2	13.2	5.7
Bacterium sp. No. 15	384	114	49.4	187	58	18.8	7.6	6.5

* The total carbon content of the culture was determined and calculated as agar, allowing 44 per cent carbon in the dry, ash-free agar.

† Residual culture was hydrolyzed by boiling for 1 hour with 2 per cent HCl; the sugar formed on hydrolysis was used as measure of residual agar.

substance. Due to the fact that agar and the other algal carbohydrates usually contain very little nitrogen, an additional source of available nitrogen, either in organic or in inorganic forms, has to be added to the cultures. This nitrogen is consumed by the bacteria and is transformed into organic nitrogenous compounds which are the constituents of the bacterial cell substance. This is clearly brought out in Table VI, where

the results of the decomposition of agar and consumption of nitrogen by the three bacteria described above are presented. One hundred-cc. portions of sterile sea water medium containing 0.6 gram air-dry agar and 0.2 gram $NaNO_3$ were inoculated with the bacteria and the cultures incubated for 30 days; the CO_2 liberated in the process of agar decomposition was determined by passing CO_2-free air over the cultures and absorbing the CO_2 in standard $Ba(OH)_2$ solution. The actual amount of

TABLE VII

Influence of nitrogen source and agar concentration upon the decomposition of agar by marine bacteria

Concentration of agar in medium	Nitrogen source	Agar left, as sugar in hydrolyzed residue				Agar consumed, as hydrolyzed sugar		
		Control	Bacterium 2 (a)	Bacterium 2 (b)	Bacterium 3	Bacterium 2 (a)	Bacterium 2 (b)	Bacterium 3
per cent								
0.5	$(NH_4)_2HPO_4$	147	0	0	147	147	147	0
0.5	$(NH_4)_2SO_4$	147	127	142	147	20	5	0
1.0	$NaNO_3$	265	127	81	265	138	184	0
0.5	$NaNO_3$	136	0	0	42	136	136	94

TABLE VIII

Utilization of mono- and di-saccharides by agar-liquefying bacteria. $NaNO_3$ as source of nitrogen, and 1 per cent carbohydrate.

Nature of sugar	Glucose				Galactose			
Organism	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3
Residual sugar	430	439	392	68	395	386	401	68
Sugar consumed	—	0	38	362	—	0	0	327
NO_3—N left	15.3	15.5	15.7	6.5	15.8	15.5	16.2	10.2
NO_3—N consumed	—	0	0	8.8	—	0	0	5.6

Nature of sugar	Mannose				Sucrose *			
Organism	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3	Control	Bacterium 2(a)	Bacterium 2(b)	Bacterium 3
Residual sugar	411	417	417	53	452	410	424	214
Sugar consumed	—	0	0	358	—	42	28	238
NO_3—N left	15.3	14.8	11.6	9.3	14.8	15.3	11.6	9.3
NO_3—N consumed	—	0.6	3.8	6.1	—	0	3.2	5.5

* Measured as invert sugar.

agar decomposed by the bacteria is greater than can be calculated from the residual carbon in the medium, since the latter includes not only the residual agar, but also certain decomposition products and the bacterial cell substance synthesized.

The nature of the nitrogen in the medium is of considerable influence in the amount of agar decomposed by different organisms, as shown in Table VII. One hundred-milligram portions of the nitrogen source were used in all the cultures. Both nitrate and ammonium salts can be utilized by the agar-liquefying bacteria as sources of nitrogen. However, in the case of the ammonium salt, the nature of the acid radical is of importance: the phosphate is as readily available to the bacteria as the nitrate; the sulfate was either not used at all or only to a very limited extent. The concentration of the agar in the medium is significant, since, in the case of one organism at least (*Bacterium* No. 3), agar was actively attacked when present in 0.5 per cent concentration but not in 1 per cent.

The ability of agar-decomposing bacteria to utilize various lower and higher carbohydrates is brought out in Tables VIII and IX. Only one of the three cultures of bacteria used in this study could assimilate all the three monosaccharides tested, namely *Bacterium* No. 3; this form does not attack cellulose and is a rather weak hemicellulose-decomposing form, as shown in Tables VII and IX. All the three organisms could utilize sucrose, the most active form being the one that could use all three monosaccharides. The two strains of the active cellulose and agar-decomposing bacterium (*Bacterium* No. 2) did not attack galactose or mannose.

As to their ability to decompose polysaccharides, the different organisms were again found to differ markedly from one another. The two strains of No. 2, the most active agar-decomposing organism, also decomposed cellulose actively, as well as starch and mannan; however, only one of the two strains (*2b*) could utilize galactan and inulin. The weak agar-liquefying organism (No. 3), which could attack monosaccharides readily, was also able to decompose most actively starch, mannan, and galactan.

The three agar-liquefying bacteria were thus found to show a very distinct specificity as regards their ability to decompose and utilize various carbohydrates. Culture No. 2 (a) attacked readily cellulose and agar, as well as starch and mannan, and sucrose only to a limited extent; it did not use glucose, galactose, and mannose, nor inulin and galactan. The other strains of this organism (No. 2b) decomposed agar and cellulose most actively, as well as inulin, starch, mannan, galactan, and were also able to make some use of glucose and sucrose. Culture No. 3 could not decompose cellulose; it acted upon agar only to a very

limited extent and under certain conditions; it was able to use, however, monosaccharides and disaccharides readily, as well as starch, mannan, galactan, and inulin. These three organisms represent only a few of a

TABLE IX

Decomposition of polysaccharides by agar-liquefying bacteria. $NaNo_3$ as source of nitrogen.

Carbohydrate	Agar				Inulin *				Starch			
Organism	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3
Reducing sugar in medium, *mgm.*	0	0	0	0	0	0	77	0	0	0	327	0
Hydrolyzed carbohydrate, *mgm.* sugar	265	127	81	265	205	204	82	189	476	63	354	0
Carbohydrate consumed, as sugar, *mgm.*	—	138	184	0	—	0	123	16	—	413	122	476
NO_3—N left, *mgm.*	15.3	10.7	9.3	11.8	—	14.8	10.2	14.3	15.8	4.9	11.1	7.8
NO_3—N consumed *mgm.*	—	4.6	6.0	3.5	—	0.5	5.1	1.0	—	10.9	4.7	8.0

Carbohydrate	Mannan				Galactan				Cellulose ‡			
Organism	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3	Control	Bact. 2(a)	Bact. 2(b)	Bact. 3
Reducing sugar in medium, *mgm.*	0	0†	23	0	0	0	0.5	0	488	335	300	495
Hydrolyzed carbohydrate, *mgm.* sugar	367	14	38	0	174	182	131	114	459	270	247	455
Carbohydrate consumed, as *mgm.* sugar	—	353	329	367	—	0	43	60	—	155	190	0
NO_3—N left, *mgm.*	—	6.9	7.4	9.3	—	14.1	9.3	10.2				
NO_3—N consumed, *mgm.*	—	8.4	7.9	6.0	—	1.2	6.0	5.1				

* 0.5 per cent used.

† In this culture practically all the mannan has disappeared, with no reducing sugar left in medium; the duplicate, however, had 255 mgm. reducing sugar left.

‡ These figures do not represent reducing sugar, but residual cellulose.

large number of bacteria present in the sea and capable of attacking various complex and simple carbohydrates.

SUMMARY

1. A study has been made of the proximate chemical composition of zoöplankton and of certain marine algæ, of their decomposition by marine bacteria, and of some of the bacteria capable of attacking certain carbohydrate constituents of marine plant life.

2. The zoöplankton was readily decomposed by bacteria in sea water and in marine mud media, with the result that more than a half of the total nitrogen present in the plankton was liberated as ammonia. Although the ammonia produced in the water and in the mud cultures was practically the same, a much greater amount of CO_2 was liberated as a result of the decomposition of the plankton in the mud than in the water medium.

3. It is suggested that the protein constituents of the zoöplankton decompose alike in the water and in the mud cultures; however, other chemical constituents of the plankton, free from nitrogen, such as certain fats, or low in nitrogen, such as the chitins, seem to undergo more rapid decomposition in the mud than in the water.

4. In the decomposition of algal material by marine bacteria, the chemical composition of the algæ is of prime importance. In the case of *Ulva lactuca*, a green alga containing about 2 per cent nitrogen on a dry, salt-free basis, active decomposition took place both in the sea water and in the marine mud cultures. In the case of the brown alga *Fucus vesiculosus*, however, containing less than 1 per cent total nitrogen, very little decomposition took place in the sea water medium, but the material decomposed very actively in the mud medium.

5. The addition of inorganic sources of nitrogen to the *Fucus* material in the sea water cultures resulted in a much more active decomposition, due to the fact that this nitrogen was required by the bacteria decomposing the *Fucus* constituents for the synthesis of their cell substance. The presence of *Fucus* material in sea water may, therefore, result in a minimum amount of available nitrogen. A definite parallelism was found between the development of the bacteria in the culture and the amount of decomposition of the *Fucus* material, as measured by CO_2 evolution.

6. Nitrate and ammonium salt were used readily as sources of nitrogen in the decomposition of the *Fucus* material by bacteria. The addition of an excess of nitrate to the culture proved to be injurious. This excess nitrate was rapidly destroyed by denitrifying bacteria.

7. The sea is found to harbor an extensive population of bacteria capable of utilizing cellulose and hemicelluloses. These bacteria represent a number of distinct types, varying considerably in their mor-

phological and physiological properties. Some of the bacteria were found capable of attacking both cellulose and agar, while others decomposed either cellulose alone or agar alone. Some of these bacteria are also able to decompose a number of other polysaccharides, as well as various mono- and disaccharides.

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ON THE PLACENTATION OF THE HARBOR PORPOISE (PHOCÆNA PHOCŒNA (LINNÆUS))

GEORGE B. WISLOCKI

DEPARTMENT OF ANATOMY, HARVARD MEDICAL SCHOOL

FOREWORD

The harbor porpoise is one of the smallest members of the order of the Cetacea. Hence its placentation is easier to study than in the larger whales where preservation and dissection are often rendered difficult by the bulk of the tissues. I had the opportunity of obtaining the gravid uterus of a harbor porpoise (*Phocæna phocæna* (Linnæus)) through the generosity of Mr. Harry Raven and Dr. Remington Kellogg. Because of compactness of size and the fact that it is in a rather good state of preservation, this uterus has afforded an excellent opportunity to study placentation in one of the Cetacea.

As far as I have been able to ascertain, the literature on this subject is not extensive and most of it is relatively old. On reviewing the literature and examining my own specimen, I was impressed by a number of points of interest which I consider to be new and sufficiently important to be set forth. In the description of the present specimen I shall not give a detailed account of what has already been observed, but will confine myself as much as possible to what is new. I am presenting a number of drawings and photographs of the specimen which give, I believe, a clearer presentation of the structure and relationships of the uterus and fetal membranes than the majority of the figures in the literature. Because of the difficulty involved in obtaining adequately fixed material, the microscopic structure of the placenta has never heretofore been very clearly demonstrated.

The literature which I have had an opportunity to consult consists of the three papers by Turner (1870, 1871, 1875) on the placentation of *Balænoptera Sibbaldii*, *Orca gladiator*, and *Monodon monoceros;* one by Chabry and Boulart (1883) on the placentation of *Delphinus delphis;* a short account by Gervais (1883) of a gravid uterus of *Pontoporia Blainvillei;* an excellent paper by Klaatsch (1886) on the placentation of *Phocæna communis;* and the work of Guldberg and Nansen (1894) on the development and structure of whales. I have not had an opportunity to consult the paper of Anderson (1878) on *Platanista* and *Orcella.* A good account of his observations is given in Klaatsch's

paper. For the still older literature I have had recourse to the contributions of Turner. In regard to the non-gravid female reproductive tract of whales, I am familiar with the paper of Beauregard and Boulart (1882), as well as the excellent studies by Daudt (1898), Hein (1914), and Meek (1918).

The Fetal Membranes

The fetus in the present specimen appears to be near term, judging from the development of its external form. It measures 92 cm. in length from tip of snout to tip of tail over the curvature of the back; 42 cm. from tip of snout to anus along the abdomen.

The general relationships of the amnion, allantois, chorion, and uterus to one another are shown in Plate I. As in all hitherto described specimens of whales, the fetus lies in the left uterine cornu. Its head is directed towards the distal end of the cornu while its tail is folded around to the right side of the body. A reversed position of the fetus *in utero* has often been observed. As is invariably mentioned, the fetal membranes extend into the right uterine cornu. In my specimen the left ovary is much larger than the right one and contains a large corpus luteum. Both Daudt and Klaatsch found the corpus luteum in the left ovary of gravid specimens of *Phocæna.* Moreover, in several non-gravid *Phocænæ* Daudt noted that the left ovary was somewhat larger than the right. To these observations it may be added that in a non-gravid specimen of the nearly related *Tursiops truncatus* I have noticed the left ovary to be larger than the right. On the contrary, Turner found a corpus luteum in the right ovary in one instance in *Orca.* It appears from these data that the left ovary of whales is the larger and is the usual site of ovulation. Moreover, nidation of the ovum is invariably such that the fetus occupies the left uterine cornu. The left-sided predominance of the ovarian tissue is in keeping with observations made on birds and some other mammals (Riddle). Hein has described characteristic differences in *Monodon* in the folds of the mucosa of the two horns in the non-gravid uterus.

As is always described, the allantoic sac, although extensive, is not so large as the amniotic sac. The amniotic sac does not in my specimen extend into the right cornu, this horn being preëmpted solely by the allantois. In the much younger specimen of *Phocæna* described by Klaatsch, amnion, as well as allantois, extends into the right cornu.

The chorion is a sac completely filling the uterus so that it is bilobed. Interesting is its degree of adherence to the uterus in various areas. In the left cornu the chorion strips away easily from the uterine mucosa. In the right cornu and, roughly speaking, in the entire area of contact of allantois and chorion it does not strip easily but clings tenaciously to the

uterine mucosa. Conversely, its easy separation from the uterus corresponds roughly to the area of contact of amnion and chorion. The arrows on the periphery of the uterus in Plate I indicate the zone of transition between adherent and non-adherent chorion. Moreover, the relationship of amnion and allantois to the degree of adherence of the chorion to the endometrium can be correlated approximately with the distribution of the allantoic vessels (Plate II). The allantois is a bilobed bag, half of it applied to the chorion on the mesial surface of the left uterine horn, the other lobe extending into and completely filling the right uterine cornu. The short umbilical cord reaches the chorion on the ventral border of the allantois in the left uterine cornu. There are four umbilical vessels—two arteries and two veins. One artery and a vein leave the cord to follow the line of chorioallantoic fusion towards the pole of the left uterine cornu, while the other artery and vein turn in the opposite direction to follow the zone of chorioallantoic fusion of the opposite lobe of the allantois. The latter artery and vein are somewhat

DESCRIPTION OF PLATES

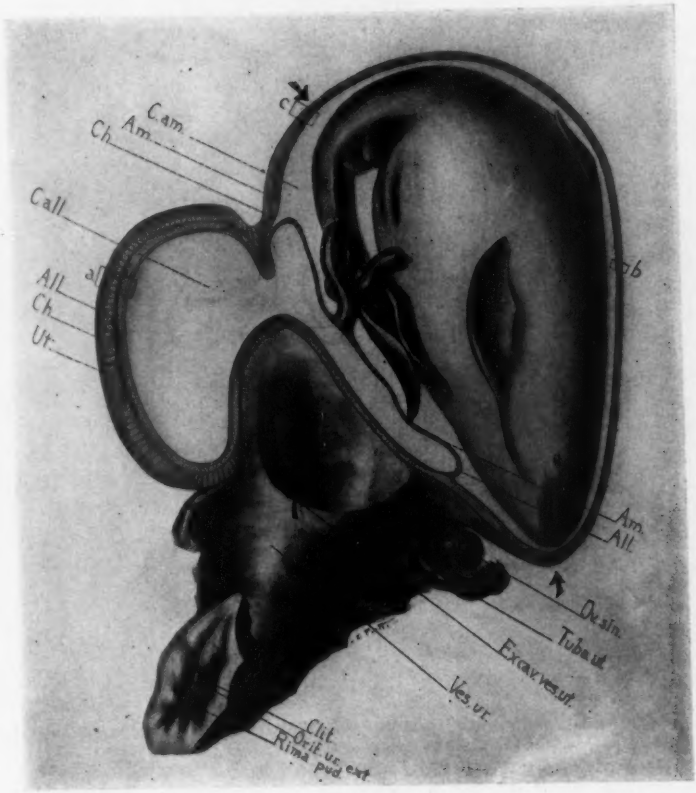
List of Abbreviations

Aa. umb.	Arteriae umbilicales
All.	Allantois
Am.	Amnion
Cav. all.	Cavum allantoidis
Cav. am.	Cavum amnii
Ch.	Chorion
Clit.	Clitoris
D. all.	Ductus allantoideus
Excav. ves. ut.	Excavatio vesico-uterina
Orif. ureth. ext.	Orificium urethrae externum
Ov. sin.	Ovarium sinistrum
Ram. vasa all.	Rami vasorum allantoideorum
Rima pud.	Rima pudendi
Tuba ut.	Tuba uterina
Ureth.	Urethra
Ut.	Uterus
Vag.	Vagina
Ves. ur.	Vesica urinaria
Vv. umb.	Venae umbilicales

Plate I

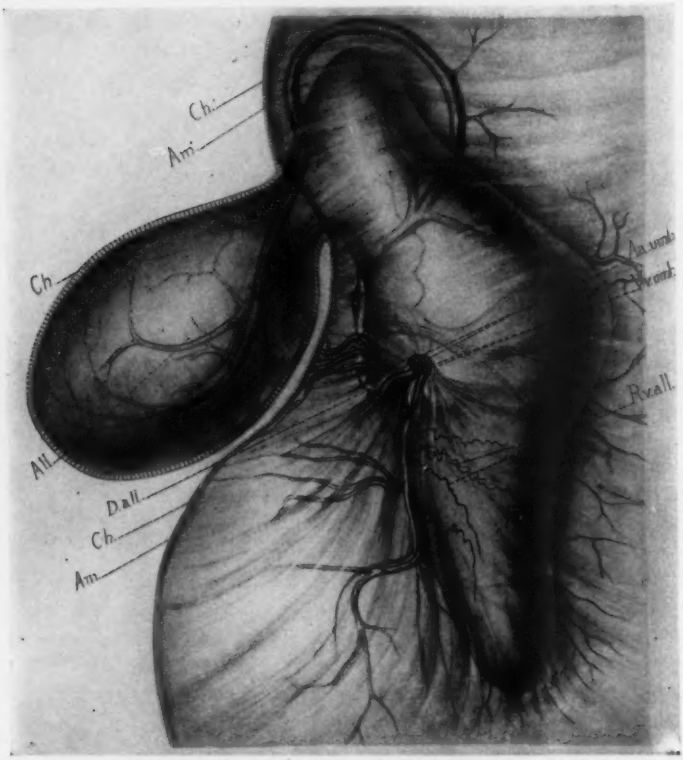
Semi-diagrammatic drawing of the gravid uterus of *Phocæna phocæna*. The fetus lies in the left uterine cornu; the fetal membranes occupy both horns. The relative size and arrangement of amnion and allantois can be seen. The allantochorion is very much thicker than the amniochorion. Three rectangles, marked *a*, *b*, *c*, indicate the sites from which blocks of tissue were taken for sectioning. The microscopic appearance of the sections from these localities is shown in Plate IV. The two arrows on the circumference of the uterus at the two poles of the left cornu indicate the transition zone from the relatively thick chorion adherent to the endometrium (allantochorion) to the thin chorion which is readily separable from the endometrium (amniochorion). × 1/7.

PLATE I


Cam.
Am.
Ch.
Call.
All.
Ch.
Ut.
Am.
All.
Ov. stu.
Tuba ut.
Excav. ves. ut.
Ves. ut.
Clit.
Orif. ur. ext.
Rima pud. ext.

larger than the preceding ones. These primary trunks give off numerous branches of succeeding orders which supply the chorion. Now it will be seen that the richest blood supply is destined for the area of

PLATE II



Semi-diagrammatic drawing of the allantoic sac to show its shape and the relationship of the umbilical blood-vessels to it. The major distribution of vessels is to the walls of the allantochorion and the neighboring zone of amniochorion. The umbilical vessels are four in number, the two smaller ones running along the inferior medial border of the allantois, the two larger ones coursing upward beneath the allantois to become distributed almost solely to the allantochorion of the right side (left of the figure). The body or left lobe of the allantois (right of the figure) has a free surface facing the observer which is not in contact with the chorion. This surface is avascular excepting a few minute vascular branches (*R. v. all.*). × 1/3.

fusion of allantois and chorion, whereas a lesser number of vessels proceed to the area of contact of chorion and amnion. The further one gets away from the allantois the less numerous the vessels become, and

that large area of easily separable contact of amnio-chorion and endometrium in the left cornu, where the uterus is greatly distended, is relatively avascular.

The intimacy of apposition of chorion to the uterine mucosa is found to correspond rather accurately to the blood supply: where the largest vessels run and the richest blood supply appears to exist the chorion is most intimately attached.

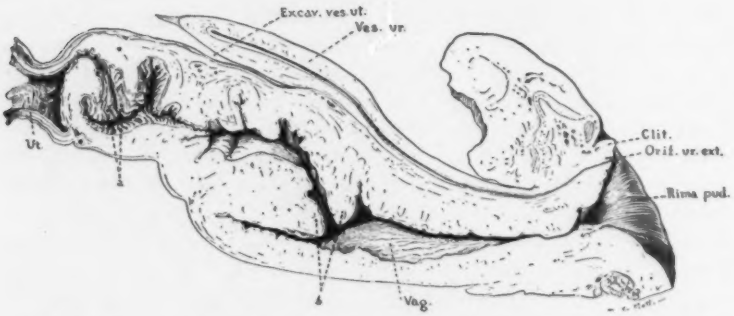
The degree of apposition of chorion to uterine mucosa is, moreover, very definitely related to morphological differences in structure of chorion and mucosa. In the relatively avascular area where the membranes strip easily the chorion is found to consist of a thin membrane which when held to the light is transparent (Plate IV, Fig. 3). Its uterine surface is almost smooth, but on closer examination is found to be covered, nevertheless, by minute villi giving it the texture of thin, rough cloth. The uterine mucosa in this area is correspondingly thin and smooth (Plate IV, Fig. 4). In a typical area of intimate connection of chorion to uterine mucosa, conditions are, on the contrary, quite different (Plate IV, Fig. 1). Traction on the chorion in such an area succeeds not in separating chorion from mucosa, but in either pulling away uterine mucosa from muscularis or the separation of chorionic mesoderm from chorionic villi. Thus in this area chorion and mucosa are rather firmly interlocked. The nature of this interlocking becomes apparent on more careful examination. It can be ascertained that the chorion is covered by branching villi which are placed at brief intervals. These structures fit into small pits with which the mucosa is studded. Separating the chorion from the mucosa is much like attempting to pull the roots of a bush out of the ground. The layer of interdigitated mucosa and chorionic villi is spongy and exceedingly thick compared with the thin chorion and mucosa in the areas of easy separation.

The area of interlocking chorionic villi and uterine mucosa passes by transition to the area where the chorionic villi become shorter and structurally simplified and separation is more easily accomplished (Plate IV, Fig. 2).

This discussion brings up the question of the bare areas or spots of which there is a variety described in various whales by previous writers (Turner, Anderson, Gervais, Klaatsch). Non-villous, membranous areas are described as existing singly or in combination in one or other species of whales in the region of the internal os or at one or both poles of the chorion. In the present specimen there is a bare area only in so far as that general area may be called bare which possesses few and short villi and is relatively avascular. My interpretation of the possible relationship of this area to the blood supply has been outlined above.

A bare area at the internal os is lacking in my specimen, although in this region the chorionic villi can be separated relatively easily from the mucosa. From the concept introduced above of the relationship of blood-supply to a relatively villous portion of the chorion, the bare areas described by previous writers in various species should be investigated again on further material. The difficulty of studying the fetal membranes in larger whales has perhaps obscured the relationships of the chorion to its blood-supply which seem rather apparent here. It should be pointed out also that upon traction artefacts giving the semblance of bare areas may be caused due to the tendency of the chorion, where

PLATE III



Drawing of the medial sagittal surface of the lower genital tract. The question of the homologies of the different segments with those of other mammals is of importance. The letters *a* and *b* indicate the structures which might be interpreted as the lips of the external os. In the text reasons are given for considering *b* as the cervical outlet, and the tract between *a* and *b* as a modified and specialized cervical region. × 1/5.

intimately interlocked with the mucosa, to cleave at the junction of chorionic villi and chorionic mesoderm or between the mucosa and muscularis. The presence of bare spots at the poles of the chorion in several of Turner's specimens led him to point out the similarity of this condition to the type of placenta referred to as *zonary*, as seen typically in carnivores. In the porpoise, however, there is no such resemblance upon which to base a relationship.

Thus, although the placenta of the porpoise is distinctly a diffuse one, there are morphologically two different areas—one, an area of intimate association of chorionic villi and mucosa which is richly vascularized; the other, an area in which the apposition of chorion to mucosa is less intimate and which is much less vascular. These areas merge imperceptibly into one another.

Microscopic Observations

The microscopic study of the mode of chorionic apposition is of considerable interest. The older writers had no means of determining the minute structure of the chorion, while the more modern investigators were dealing more often with poorly fixed material from larger whales in which the epithelium was largely destroyed by post-mortem changes, or were describing the gross relationships only. Thus in the descriptions and figures of Turner, Klaatsch, and Guldberg and Nansen one is left with considerable doubt as to the finer structure of the chorion and uterine mucosa. In the present specimen the tissues have been sufficiently well fixed to give a fairly adequate picture of the histology.

It was pointed out above that the chorion could be divided roughly into two areas, one of intimate attachment of the chorion to the endometrium, one of extremely loose attachment. It was stated that the area of intimate fusion coincided approximately with the area of the allantochorion; that of less intimate association roughly with the extent of the amniochorion. This separation into two areas is amply borne out by microscopic examination of sections taken from the two regions. Figure 1 is taken from the region of intimate interdigitation of chorion and allantois and shows the broad zone of interpenetration of the spongy endometrium and the villous chorion. Separation of the two membranes is possible only by tearing. In Plate I the place where this section was taken is shown by a rectangle marked *a*. In contrast, Figs. 3 and 4 of Plate IV show the chorion and endometrium in the other region where contact of chorion and endometrium is slight. The site from which the sections were taken is indicated in Plate I by a rectangle marked *b*. It will be seen in the figures that the chorionic villi are entirely absent or stunted in this region and that the endometrium is insignificant in its proportions. Continuing the comparison of these sections with the previous one, it will be observed that the uterine glands are extremely attenuated in contrast to their profusion in the former region. Finally, Fig. 2, Plate IV, shows an intermediate or transitional area between the two regions (rectangle *c*, Plate I). Here the interdigitation and proliferation of chorion and endometrium are less marked than in Fig. 1 taken from the zone of maximum growth.

The main question of interest, however, that can be answered by examination of the present sections is whether the porpoise's diffuse placenta is wholly an epitheliochorial one or is in part a syndesmochorial one. Examination of the present sections indicates that the former is true—that both chorion and uterine mucosa retain everywhere their epithelial covering (Figs. 5 and 6). It can be seen in the area of maxi-

mum proliferation of the chorion that both uterine and chorionic epithelium (Fig. 6) are separate and intact layers. The former is composed of flattened, endothelium-like cells with sharply defined boundaries. The chorionic epithelium, on the other hand, is made up of less regularly arranged cells with rather indistinct boundaries suggesting that the epithelium may be in part or wholly syncytial (Fig. 6).

The tufted chorionic villi are applied loosely to the mucosa, excepting at their distal ends which are free, resulting in a considerable separation between the endometrium and the tips of the chorionic villi (Fig. 5). These spaces do not appear to be artefacts, due entirely to shrinkage in fixation. Instead, they have the appearance of being naturally existing spaces filled with embryotrophic material derived from the uterine epithelium or from the abundant uterine glands. The secretion occupying these spaces appears to be formed of amorphous granules. Moreover, the uterine epithelium in this fundic region of the endometrial crypts is more cuboidal in shape than that of the remainder of the crypts where endometrium and chorion are in closer approximation. The secretion which accumulates in these pockets at the tips of the villi is derived presumably in part from the actively secreting uterine glands. The cells constituting the uterine glands appear to be large and swollen as though they were actively secreting. As is well known from the studies of Turner and Klaatsch, several of these uterine glands unite to empty

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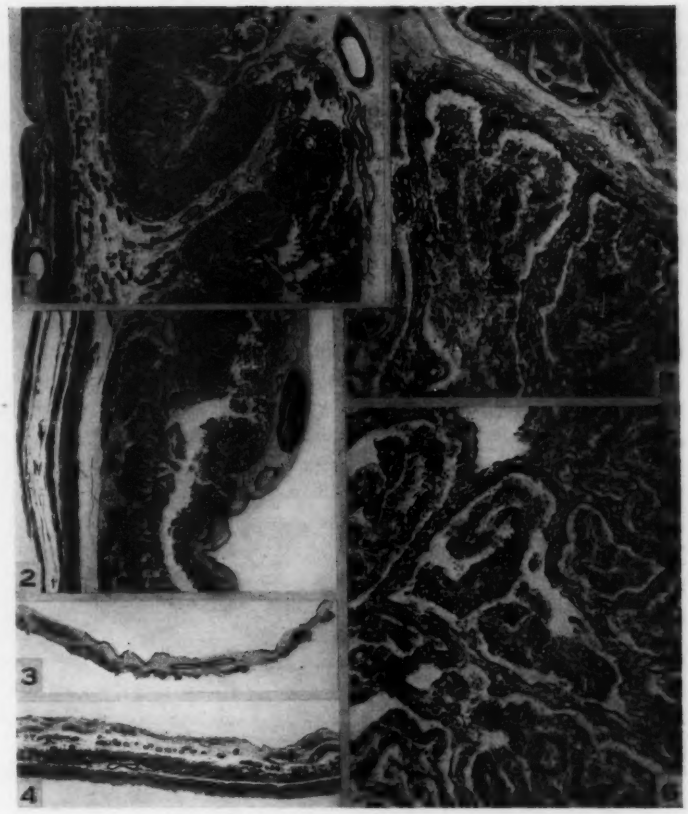
PLATE IV

FIGS. 1 to 4 show typical sections through the uterus and placental membranes from the regions designated *a*, *b*, and *c* in Plate I. Note that in Fig. 1 (area *a*, Plate I) endometrium and allantochorion are intimately united to constitute a broad placental zone, while in Figs. 3 and 4, which are from the amniochorion and uterine wall respectively of area *b*, Plate I, the chorion and endometrium are simple in structure and consequently are not intimately united. Figure 2 shows an area (*c*, Plate I) of transition midway between areas *a* and *b*. A tendency for separation of chorion and endometrium to occur is apparent. Note that the zone of endometrial glands undergoes a similar simplification from the villous to the non-villous placental area. Figures 1 to 4 are all taken at the same magnification. × 8.

FIG. 5. Higher magnification of a part of Fig. 1. The basal part of the placenta is shown, illustrating the tips of the fetal villi ending free in the endometrial crypts. In the spaces between the tips of the villi and the basal endometrium there is a profuse secretion of uterine milk. The endometrium is composed of a single unbroken layer of cells, cuboidal in the basal region, flattened on the endometrial septa. The covering of the fetal villi is everywhere present, but irregular in thickness, suggesting that the surface layer is syncytial. × 103.

FIG. 6. A photograph of the middle of the placental zone from the area shown under low magnification in Fig. 1. Note the septa or trabeculæ composed of endometrium which enclose spaces or crypts in which the chorionic villi lie. The endometrial trabeculæ are lined by extremely flattened epithelial cells, while the fetal villi are clothed by chorionic epithelium of a syncytial nature and irregularly thickened. × 103.

PLATE IV



on the surface by a common outlet which can be seen grossly as minute, whitish flecks designated as areolæ scattered irregularly over the surface of the mucosa when it is viewed stripped away from the chorion. In my experience with the porpoise these are easily seen with the naked eye in the extensive area of mucosa in the left uterine cornu in loose apposition to the chorion. In the area, on the contrary, in which the interlocking of chorion and endometrium is intimate, where the mucosa is thickened and more or less honeycombed for the reception of the chorionic villi, the areolæ are invisible. This, I believe, should not cause surprise for the glandular orifices empty into the deeper, concealed parts of the recesses created by the markedly proliferated endometrium. On the surface actually, after the chorion has been pulled loose, only the crypts are seen which provide for the penetration of the chorionic villi into the spongy endometrium. The areolar areas are invisible. This circumstance has been overlooked by Klaatsch who, I believe, confuses the areolar openings and the mouths of the endometrial crypts which are of a different nature.

In the chorion of my specimen I have looked in vain for corresponding chorionic areolæ or vesicles such as characterize the chorion of the sow which are described as present in specimens of various cetaceans investigated by Turner and Klaatsch. It appears in my specimen, in contrast to the sow, as though the uterine secretion after being discharged from the uterine glands undergoes absorption in the spaces or recesses just described between the tips of the chorionic villi and the funduses of the endometrial crypts, instead of as in the sow, in which small areolar pockets reserved for the absorption of embryotrophe indent the simple chorion, leaving chorionic folds around them, the latter subserving the more intimate union necessary for gaseous exchange. In the porpoise it seems apparent that the tips of chorionic villi dip down into the spongy endometrium to tap the reservoir of maternal embryotrophe situated at the bases of the endometrial trabeculæ, whereas the superficial zone of the endometrium is designed for that more intimate apposition of the chorion, providing for the more direct metabolic exchange.

To continue the discussion of the detailed anatomy of the membranes, Turner and Klaatsch make note of a definite pattern of the chorionic villi, reporting that the villi tend to be arranged or grouped in a linear way often conforming to the course of larger blood-vessels or folds of the chorion. Of this I am able to make out little or nothing in my specimen. When larger areas of the chorion are viewed, one does notice a certain irregularity or patchiness of the velvet-like villi, but this unevenness of height and density does not conform to any definable pattern

The main differences between the height and density of the villi in my specimen are related solely to the two major areas of the chorion emphasized in the previous account.

A few more words should be devoted to the allantois. As has been said, it is a bilobed bag largely in contact with the chorion. The left lobe, however, is fused over about half of its surface with the amnion. The allantois and amnion constitute together a thick transparent membrane separating allantoic from amniotic fluid. In this membrane a few blood-vessels are present which are given off as slender branches from the larger allantoic vessels supplying the chorion (*R. v. all.*, Plate III). In the allantoic fluid are a number of small, free bodies which are brown, irregular in outline, and of the consistency of gum. On the amnion, more particularly that portion fused with the allantois, there are numerous minute, brownish elevations, the carunculæ of previous observers. These structures, which there is no necessity of describing in detail, are especially abundant here as well as on the umbilical cord.

The Umbilical Cord

A few observations of interest have been made on the umbilical cord. A detailed account of structures already well known will not be undertaken. I have paid no particular attention to the epithelial covering of the cord which possesses characteristic epithelial thickenings, so-called carunculæ which have been described in detail for *Phocæna* by Klaatsch and for various other species by Turner.

A cross-section through the middle of the umbilical cord of *Phocæna* reveals two arteries and two veins. Besides these the cross-section shows several duct-like structures (Plate V, Fig. 10). A decision as to the nature of these several ducts offers difficulties, of which Klaatsch was aware. First of all there is a large epithelial-lined duct in the center of the cord which by dissection can be traced in both directions, and is found to communicate with the allantois at one end and with the urachus at the other—evidently the allantoic duct (Fig. 10, *a*). This is lined by cells of variable height: in part they are cuboidal, in other part lower than cuboidal (Fig. 11). Two similar duct-like spaces lined by a similar type of epithelium are found opposite to one another on the periphery of the cord (Fig. 10, *b*, *c*). In addition to these, several smaller cleft-like spaces lined by epithelium are visible. All of these spaces exhibit the same type of epithelium which suggests that they are related to one another. On tracing the two larger of the clefts by dissection of the cord no final answer as to their relationships is obtained. Nevertheless, in two places they appear definitely to join and to become part of the centrally located allantoic duct. Towards the placenta the two clefts

appear to lose themselves in connective tissue. Towards the umbilical ring one of them seemingly joins the allantoic duct. I interpret these clefts as diverticulæ of the urachus or allantoic duct. Near the umbilical ring the allantoic duct is an extensive structure composed of clefts which extend widely around the circumferences of the blood-vessels. It is not unlikely that the aforementioned clefts, which farther out on the cord are seemingly separated from the allantois, are parts of the allantoic duct which are partially separated by septa from the main duct and which communicate with the main duct at one or more points. Klaatsch in his younger specimen found similar evidence of the occurrence of diverticulæ of the allantoic duct. Nothing short of study of serial sections through the cord of *Phocæna* would reveal the exact configuration of the allantoic duct.

In my own material I find no signs of the vitelline duct or remnants of it. In Klaatsch's younger specimens he interpreted certain epithelial structures as the remains of it.

Another striking characteristic of the cord of *Phocæna* mentioned by Klaatsch and especially clearly visible in my own specimen is the presence of bundles of smooth muscle running longitudinally in the stroma of the cord (Fig. 11). Klaatsch interpreted these unusual bundles as remnants of the yolk sac or in part as off-shoots from the muscular tunics of the umbilical vessels. Neither of these explanations accounts, I believe, for the presence of so many stout, rather scattered bundles of muscle fibers. What their nature may be must remain undetermined for the present.

Finally I wish to make a few remarks upon a further interesting observation by Klaatsch. He mentions, as a puzzling feature of the

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PLATE V

FIGS. 7 and 8. Photographs showing at low magnifications the topography of the placenta in the region of area *a*, Plate I. The irregular crypts demarcated by endometrial septa and containing the chorionic villi can be seen. × 47.

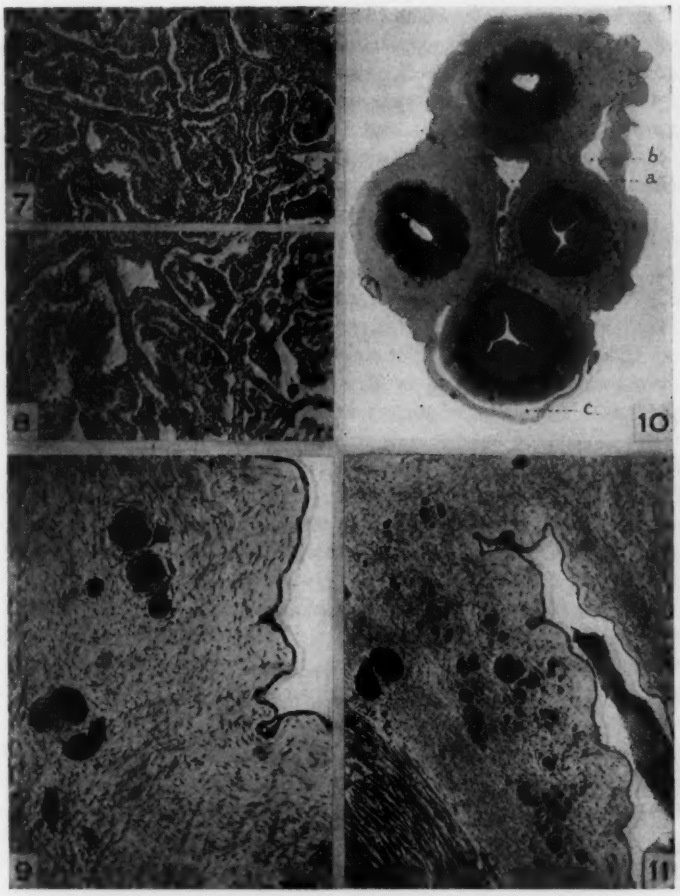
FIG. 9. A view of the stroma of the umbilical cord, showing a number of small blood-vessels by which the stroma is vascularized. × 47.

FIG. 10. Cross-section of the umbilical cord midway between the umbilicus and the allantois, showing the four umbilical vessels. Three clefts, one in the center and two situated peripherally marked *a*, *b*, and *c*, indicate the allantoic duct (*a*) and its diverticulæ (*b* and *c*). The small black dots scattered throughout the stroma of the cord are for the most part small arteries and veins which constitute a vascular bed in the stroma of the cord, similar to the vessels shown under higher magnification in the previous figure. × 8.

FIG. 11. Higher magnification of a part of the umbilical cord adjacent to the allantoic duct showing bundles of smooth muscle fibers scattered through the stroma and bearing no apparent relationship to the musculature of any of the four major umbilical vessels. On the left of the figure two larger, very black areas are small blood-vessels supplying the stroma. × 47.

cord of *Phocæna,* the presence in the stroma of several small arteries and veins, evidently branches of the umbilical arteries and veins. As to the significance of these small vessels in the cord, Klaatsch makes no remarks. In my own specimen these small vessels in the stroma of cord

PLATE V



are very distinct and much more abundant than one would have supposed from Klaatsch's observations (Figs. 9 and 10). Indeed the stroma of the cord appears to be richly vascularized at a period near the termination of pregnancy. This vascularization of the cord at such an

advanced age is of interest to me because of my observations of the cords of other animals. The umbilical cord of man and other primates is said to have no blood-vessels other than the umbilical arteries and veins; the stroma of the cord is supposedly avascular, possessing no capillaries. Only at the period of blood-vessel formation in the body-stalk is there a vascular plexus in the cord. This period is brief, the vessels of the cord being reduced early to the several major arteries and veins. According to my observations many mammals are quite different from man in that the connective tissue of the fully developed cord at the height of gestation possesses an extensive vascular bed. This blood vascular bed is independent of and has nothing to do with the vitelline blood-supply which in some animals may be extensive and coexistent with it. Grosser (1927) makes no mention of this subject; Jordan (1919), however, describes vascularization of the connective tissue in sections of the umbilical cord of the pig. Other than Klaatsch's remarks on the porpoise and Jordan's observations upon the pig, no further study or discussion of this matter appears to exist in the literature. My first experience in regard to it was with pig fetuses in which, at stages of from 100 to approximately 250 mm. crownrump length, I have been able to inject a rich set of blood-vessels in the stroma of the cord. In cleared specimens I have observed the origin of these vessels in the pig as minute arteries given off at frequent intervals from the umbilical arteries. Small veins similarly join the umbilical veins at short distances apart. The entire cord is vascularized in this manner. On observing sections of the cords of a variety of other mammals I have found evidence of similar vascularization of the tissue composing the cord. Amongst them is the cord of *Phocæna*.

I have made two groups of the animals which I have investigated. I was careful in each instance to take the section from the middle of the cord well away from its attachment to the placenta. Those which have a vascularized stroma of the umbilical cord are:

	Species	Stage examined
Pig	*Sus scrofa*	100–250 mm. C. R.
Hippopotamus	*Hippopotamus sp.?*	Term
Peccary	*Pecari angulatus bangsi*	47 mm. C. R.
Porpoise	*Phocæna phocæna*	Near term
Hyrax	*Proacavia capensis*	147 mm. C. R.
Sloth	*Bradypus griseus griseus*	215 mm.
Agouti	*Dasyprocta punctata isthmica*	126 mm. C. R.
Capybara	*Hydrochærus capybara*	200 mm. C. R.
Galeopithecus	*Galeopithecus volans*	Middle gestation

Those which have not a vascularized stroma of the cord are:

Tapir	*Tapirella bairdii*	190 mm. C. R.
Galago	*Galagoides demidoffi*	25 mm. C. R.
Marmoset	*Œdipomidas geoffroyi*	47 mm. C. R.
Howling monkey	*Alouatta palliata*	Middle gestation
Spider monkey	*Ateles geoffroyi*	Term
Gibbon	*Hylobates pileatus*	Near term
Chimpanzee	*Pan paniscus satyrus*	Near term

Thus it will be seen that outside of the Order Primates the majority of the animals examined possess well-vascularized umbilical cords and *Phocæna* belongs to the latter category. I hope to publish elsewhere the results of a further study of this matter. What the functional significance of the possession of a vascularized umbilical cord may be remains obscure. It may have some bearing on the mode of formation and changes in quantity of the amniotic fluid.

Besides this capillary blood supply to the umbilical cord of *Phocæna*, the question arises as to whether there are lymphatics in the cord. I mention this because Anderson and Klaatsch describe lymphatics in the cords of their specimens of whales, the latter giving a figure of an endothelial-lined channel which he believes is a lymph-channel. He says in another place in his paper, however, that the epithelial linings of the several structures in the cord were so poorly preserved that he was uncertain as to their morphology. This makes me doubt the accuracy of his observation on the endothelium of the supposed lymphatics. Moreover, he speaks of the anatomical continuity of this lymph channel with tissue spaces. On examination of my own specimen I am led to believe that he and Anderson erred in the identification of lymphatics. I find nothing that resembles true lymphatics. However, without resorting to injection methods to fill lymph vessels, it is impossible to dismiss with certainty the possibility of their existence in a given tissue.

The Genital Outlet

A sketch is presented of the mid-sagittal view of the genital outlet of the present specimen (Plate III). The topography of the organ is of some interest and deserves a brief discussion. Daudt and Meek both describe the vagina of the porpoise as consisting of two segments—a lower segment that is relatively straight and smooth, and an upper segment of about equal length that is partially subdivided by the presence of several prominent bulging folds. Such an assumption on their part defines the os uteri as a short segment adjacent to the uterus indicated in the present sketch by the letter *a*, Plate III. An alternative interpretation of the homologies of the structures appears to me to bear examination. The os uteri externi could be homologized instead with

the bulging lips marked *b* in the drawing, in which case the cervical segment would include all of that segment designated as the upper part of the vagina by Meek and Daudt. In favor of this assumption is the fact that there are well-marked fornices at this point and a rather sharp demarcation between the musculature above and below it, as is shown in the drawing. Moreover, the upper third of this segment, belonging to the vagina according to Meek and Daudt, is clearly glandular as microscopic examination reveals. These glands are abundant and deep, and their secretion completely fills the lumen of the region in question, between *a* and *b*, with a gelatinous mass. This jelly was removed before the sketch was made. Thus the walls of the space immediately caudal to the folds, termed the *spermothecal recess* by Meek, are extremely glandular and the lumen is distended with jelly-like secretion.

To answer the question raised, in view of our almost complete ignorance of the comparative anatomy of the lowermost uterine segment, is impossible. The subject of the homologies of this segment in the mammalian series is one that is almost untouched. By comparison with the human, which is at the moment almost the sole basis for comparison, little help is obtained. Nevertheless, it is known from studies on the macaque (Joachimovits, 1928; Wislocki, 1932) that the cervical segment may be a much more complicated succession of folds than is ordinarily realized. On the other hand, in other animals it may be much less conspicuous than in the human, as for example in the gibbon (Wislocki, 1932). Concerning the question of whether in the Cetacea the elaborate sets of folds at the junction of uterus and vagina should be regarded as a part of the former or of the latter, extreme caution should be exercised until further comparative morphological studies of the subject have been undertaken. In the one instance the cervix would have to be regarded as extremely reduced; in the other event, it would have to be looked upon as a highly developed and specialized structure.

Summary and Conclusions

Examination of a rather well-preserved specimen of one of the smallest species of whales (Phocæna phocœna) demonstrates that near term the placenta is diffuse and is of the epithelio-chorial type. Although diffuse in character, the chorion can be divided at this stage into two rather well-defined areas; the first, an area of intimate interdigitation of branching villi and proliferated endometrium corresponding in the main to the region of allantochorionic fusion; the second, an area of stunted villi and reduced endometrium corresponding roughly to the area of amniochorionic fusion. These areas can be related to some extent to

the allantoic blood supply, which is much more profuse in the region of the allantochorion than in the region of the amniochorion.

In spite of the fact that the branching chorionic villi are intimately interlocked in the allantochorionic area with crypts of the uterine mucosa, the endometrium, as well as the chorion, retains its complete integrity by virtue of the survival of intact epithelial coverings. In the case of the endometrium the epithelium is cuboidal in the fundic parts of the endometrial crypts, whereas it is flattened in the outer and more extensive parts of the crypts. The chorionic epithelium, on the contrary, although forming a complete investiture of the villi, is interpreted as being syncytial in character. Thus the placenta is of the epithelio-chorial type, as described in the sow, horse, tapir, hippopotamus, camel, *Manis,* and the lemurs (with the possible exception of *Galago*). The interlocking of chorionic villi and endometrium is, however, much more complex than in the sow, being more like that described in the horse. Whether the attenuated endometrium in the amniochorionic area of the porpoise bears close analogy to the horse, in which widespread degeneration of the endometrium is said to occur in the fourth and fifth months, cannot be ascertained without access to further stages of the porpoise. Be that as it may, the porpoise placenta does not appear to be of the syndesmochorial type which occurs typically in many ungulates with cotyledonary placentæ. It appears inconceivable, however, that at parturition the fetal and maternal tissues, which are intimately interlocked in the area of the allantochorion, are destined to separate without a certain amount of damage to the chorion and endometrium. Either the endometrium must be torn away to some degree or the chorionic villi must become torn off and retained to a certain extent. A separation without trauma to the tissues appears unlikely.

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POLYMORPHISM AND METHODS OF ASEXUAL REPRODUCTION IN THE ANNELID, DODECACERIA, OF VINEYARD SOUND

EARL A. MARTIN

DEPARTMENT OF ANATOMY, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK CITY

Remarkable diversity in the reproductive phenomena of the European variety of *Dodecaceria concharum*, an annelid worm, has been shown by the work of Caullery and Mesnil, and Dehorne. The first authors reported three forms which they designated as *A*, *B*, and *C*. Form *A* was sedentary, parthenogenetic, and viviparous. Form *B* was epitokous, pelagic when mature, and both males and females occurred in approximately equal numbers. Form *C* showed a tendency to become epitokous but was thought to be sedentary when mature. In this form only females were found, and since large mucous glands were present near the gonoducts it was concluded that the form was parthenogenetic and oviparous. Form *A* was most numerous, form *B* occurred in much smaller numbers, and form *C* occurred very rarely.

In 1924 Dehorne reported that a very primitive type of asexual reproduction occurs in form *B* described by the previous workers. In this process the metameres in the middle region of the body become constricted at the annuli and break away into single units. These free metameres regenerate from the anterior surface new head and body segments and from the posterior surface a new anus and body segments. The new individual then becomes a young *Dodecaceria*. More recently, in the fall of 1932, Dehorne has modified this description and reports that the original segment does not actually become incorporated into the new individual but that when the anterior and posterior regenerating ends reach a certain stage they separate at the junction of the new material with the old metamere and by further regeneration form two complete individuals. The original segment may repeat the process of regenerating head and tail portions a second time and these again break away, leaving the old metamere which then probably dies. This breaking up into segments or autotomy of the middle region in the original adult leaves the anterior piece without a tail end and the posterior piece without a head end. Dehorne, 1927, reported that each of these two portions regenerated its missing end and that some of the individuals produced in this manner may give rise to the sexual form *B*.

The *Dodecaceria* of the Woods Hole region were originally described by Leidy and Verrill. In 1855 Leidy recorded a species of annelid found burrowing in dead coral (*Astrangia danae*) at Point Judith which he named *Naraganseta coralii*. This species was subsequently placed in the genus *Dodecaceria* by Verrill. In 1879 Verrill described a species collected in the Bay of Fundy and named it *Heterocirrus fimbriatus*. He reported that this species occurred along with *Dodecaceria concharum* and was related to it. Caullery and Mesnil later called attention to the fact that *Heterocirrus fimbriatus* resembled closely the form *B* of *Dodecaceria concharum* and suggested that it belonged to the genus *Dodecaceria*.

Several years ago the writer found that *Dodecaceria* occurred in abundance in the nodules of encrusting Bryozoa in Vineyard Sound.

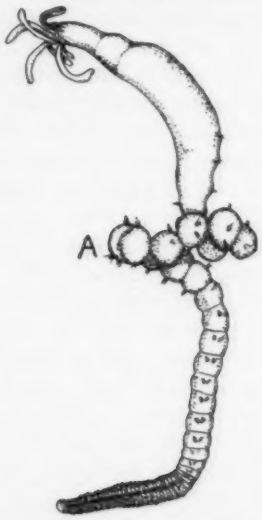

FIG. 1. *Dodecaceria fimbriatus* in asexual reproduction. Region *A* breaking into single metameres by segmental constrictions.

The scant references concerning the American species, together with the interesting phenomena described by Caullery and Mesnil and especially their observation that *Heterocirrus fimbriatus* resembled the form *B* of *Dodecaceria concharum*, led me to begin an investigation of the *Dodecaceria* at Woods Hole. After a preliminary survey intensive investigation was started in July, 1932. At present, collection of material is being made at frequent intervals in order that the life cycle may be determined throughout the year.

Early in this investigation the *Heterocirrus fimbriatus* of Verrill was identified and its resemblance to the form *B* of *Dodecaceria concharum* was found to be so striking that thus far it has been impossible to distinguish differences between them. They appear identical in the development of the eyes, the atrophy of the palps, and the setigerous pattern. A more critical study of the setæ in the two is being made, but on preliminary study similarity extends even to the possession of a tooth at the base of the excavation in the spoon-shaped setæ.

Caullery and Mesnil have described the presence of a gregarine parasite, *Gonospora longissima,* in form *B*. This parasite may be observed as a white mass under the yellow integument. When mature specimens of form *B* are disturbed in the *Lithothamnion* (calcareous algæ) they swim violently, shedding their eggs or sperm, and when empty the gregarines can be seen as long white threads extending out of the gonoducts. The behavior of *Heterocirrus fimbriatus* at maturity is

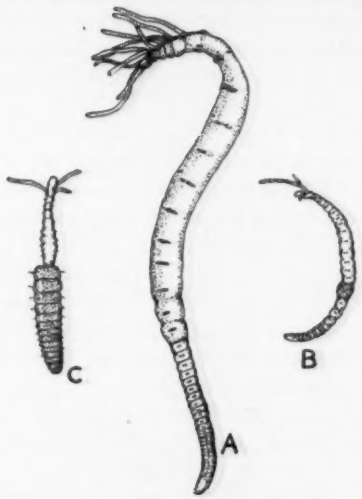

FIG. 2. *A*. Anterior end of worm regenerating a tail after autotomy. *B*. Single segment that has regenerated 12 segments anteriorly and 11 segments posteriorly. *C*. Posterior end of original worm that has regenerated 9 segments anteriorly.

identical with the description of form *B*, and a gregarine parasite is present which also appears to be identical with the *Gonospora longissima* of form *B*. These observations demonstrate that the *Heterocirrus* of Verrill belongs in the genus *Dodecaceria*. It has not been possible to distinguish *fimbriatus* from *concharum,* but since the existence of the

same species on opposite sides of the Atlantic seems improbable, decision is reserved on this point.

Asexual reproduction began in my material during the latter part of the summer. I was unaware of the work of Dehorne and my conclusions thus far have been formed independently of Dehorne's papers. Professor Caullery, during a recent visit to this laboratory, gave me the references to Dehorne's publications.

The phenomenon of asexual reproduction in the American form (Figs. 1 and 2) is essentially the same as described by Dehorne, and those differences that do exist between his observations and mine may be resolved by further study. For example, I have observed, contrary to Dehorne's last report, that single segments may give rise to one individual without further autotomy, in which case the original segment is incorporated into the new individual. This is in line with Dehorne's earlier idea. It has been possible to make this certain since the original segment can be identified in these individuals by a darker band of pigmentation (Fig. 3).

My observations on the further autotomy of the regenerating anterior

FIG. 3. Young individual derived from a single segment. Note dark band which marks the original metamere.

and posterior ends are not yet complete. I am sure, however, that this phenomenon also occurs in the American form and that it is probably quite extensive since there are relatively few of the young *Dodecaceria* which possess the dark band of pigmentation referred to above. Reliable data on this point can be derived only from newly collected material and not from specimens living in laboratory vessels. It is found

that when regenerating segments are removed from the bryozoan nodules in which the worms live and are placed in glass dishes, mucus is secreted which accumulates at the junction of the old segment with the regenerating tissue, and there is evidence that this increases the tendency to autotomize at this point.

Since Caullery and Mesnil did not find asexual reproduction in specimens taken from the *Lithothamnion* at Cherbourg and since Dehorne did find both the pelagic form *B* together with asexually reproducing individuals in the marl at Le Portel, Dehorne concluded that the asexual reproduction may have been caused by chemical differences in the material in which the worms were burrowing. The fact that, as my study shows, the phenomenon of asexual reproduction in *Dodecaceria* has a wider distribution than the French workers thought seems to make this conclusion doubtful.

I have recently found indications that the anterior end of the asexual parent produces the epitokous form *B*, thus confirming the report of Dehorne. One specimen, recently found regenerating the posterior end after autotomy, contained the gregarine parasites mentioned above. Another specimen was observed which had developed pelagic setæ and in which the posterior end showed evidences of having recently been regenerated. Further observations on this point are under way.

A careful search has been made for forms resembling *A* and *C*, but thus far there is no evidence that they exist on this side of the Atlantic. Early in my investigation many specimens collected were thought to be form *A*, but it was found that they differed in their morphology from all forms described by European authors. These specimens have been identified as *Dodecaceria coralii*. This species is larger than the European *Dodecaceria*, which never possess as many as 100 segments, while the larger specimens of *Dodecaceria coralii* exceed 100 and may reach as many as 145 segments. This is the most abundant species in Vineyard Sound and the point of chief interest is that it is sedentary throughout life, showing no indications of an epitoke, and that both males and females occur. It is not viviparous.

Caullery and Mesnil, although, as they pointed out, there was some evidence to the contrary, concluded that the forms *A*, *B*, and *C* were members of one polymorphic species. The close similarity between forms *B* on opposite sides of the Atlantic in both their sexual and asexual phases indicates close relationship. This, together with the fact that the forms *A* and *C* do not occur on this side of the Atlantic, has led the writer to believe that the ensemble *A*, *B*, and *C* which Caullery and Mesnil have classed as a polymorphic species is actually a group consisting of more than one species. On his recent visit Professor Caullery

informed me that work now being done in Europe has led him to the same opinion.

The tentative conclusion concerning the American forms is that two species exist, *Dodecaceria coralii* and *Dodecaceria fimbriatus*, the latter being polymorphic, consisting of sexual and asexual individuals.

The reproductive phenomena displayed by *Dodecaceria* may be arranged in a series in which pelagic epitokous forms occur at one end, producing eggs and sperm, while at the other end reproduction is effected by as primitive a form of asexual reproduction as is known in the phylum. It is interesting to note that the polymorphic form *B* occupies both ends of the series.

Reproductive Series in Dodecaceria

1. Eggs and sperm shed by pelagic epitokous males and females. Form *B, Dodecaceria concharum* and *D. fimbriatus*.

2. Eggs and sperm produced by sedentary atokous males and females that are oviparous. *D. coralii*.

3. Parthenogenetic eggs produced by sedentary epitokous females that are oviparous. Form *C, Dodecaceria concharum*.

4. Parthenogenetic eggs that develop in the cœlom to a larval stage (viviparous). Form *A, Dodecaceria concharum*.

5. Asexual reproduction by separation of single metameres which regenerate complete worms. *D. fimbriatus*.

6. Asexual reproduction by secondary autotomy of regenerating segments. Form *B, Dodecaceria concharum* and *D. fimbriatus*.

Close similarities among the vegetative structures make this a valuable group in which to study the evolution of different methods of reproduction. In spite of wide geographic distribution of these annelids their environmental conditions are similar wherever they occur. The similarity that exists between the biological associations in the *Lithothamnion* at Cherbourg (Caullery and Mesnil) and the association of species in the bryozoan nodules of Vineyard Sound is striking. It is suggested that the uniformity of the environment has decreased the effectiveness of selection on vegetative characters of these worms, while the burrowing habit has increased the effectiveness of selection on possible methods of reproduction in the burrows, thereby avoiding losses incident to pelagic spawning. I infer that asexual reproduction in *Dodecaceria* is a secondary phenomenon.

The identity between *Dodecaceria fimbriatus* and the form *B* of *Dodecaceria concharum* on opposite sides of the Atlantic presents an interesting problem in geographic distribution. If *Dodecaceria fimbriatus* and the form *B* of *Dodecaceria concharum* are different species,

which would seem probable, they are so closely related that they must have arisen in a common region. There is small chance of continuity across the abysmal regions of the Atlantic, nor is the duration of life of the pelagic form sufficient for it to be carried any great distance. *Dodecaceria* has been reported as occurring on the piles in Nantucket Harbor, which suggests the possibility that the larva of the pelagic form may live on the encrusted bottoms of ships and in this manner be carried across the Atlantic. However, since *Dodecaceria* is a primitive genus and also since the calcareous materials in which they occur are old geologically, it is probable that these worms have existed in their present habitat for a long period of time. It is planned during the coming summer to learn, if possible, whether there are differences among forms of *Dodecaceria* which range north and south of Cape Cod.

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STUDIES ON THE CILIATES FROM SEA URCHINS

I. General Taxonomy

PHILIP B. A. POWERS

Zoölogical Laboratory, University of Pennsylvania

Introduction

Five (+ 2?) species of ciliates are found infesting the intestinal cæca of *Strongylocentrotus dröbachiensis* (O. F. Müller). In studying these ciliates it was of interest to examine allied species known to be endemic to sea urchins of other localities.

The data growing from these investigations can best be presented in the form of a series of papers of which this is the first. The subsequent papers will (where such studies may be of value) present observations on the detailed morphology of individual or closely allied species. This first report is in the nature of a general survey covering 14 species of ciliates found infesting 3 species of sea urchins.

Materials and Methods

In Table I are shown the various species of sea urchins examined, their source, and the number of species of ciliates comprising their infestation.

In addition to the above, species of *Arbacia* from Cape Cod, the Bay of Naples, and from Beaufort, N.C., were examined for ciliates but with negative results.

When collecting samples of the intestinal contents of sea urchins, it was found advisable to take precautions to prevent any possible contamination of the sample with possible chance mixtures of free-living ciliates. It was found that by rinsing the sea urchin in sterile fresh water and then using clean instruments in subsequent operations, possibilities of chance contamination were eliminated. Observing these necessary precautions, the technique used in this study was essentially that of Lynch (1929): Freshly collected and healthy sea urchins were opened by cutting the test in half with heavy scissors. The perivisceral fluid was drained out and the intestine removed to a clean dish, opened, and its liquid content collected with a sterile pipette and either studied immediately, or preserved in filtered sea water at 10° C., or the material was fixed as smears on coverslips. Material was also preserved in bulk by spurting the intestinal fluid into small flasks of warm fixative.

The most successful fixatives used were Schaudinn's, Bouin's, or Flemming's fluid without acetic acid (F. W. A.). The fixed material was studied in the form of whole mounts or as sections 7 to 10 μ thick. The principal stain employed was iron-alum hæmatoxylin. Mayer's hæmalum, as well as Lynch's (1929) method of using carmine and indulin were found very helpful in confirming observations. Feulgen's nucleal reaction was used as a final check on the nuclear structures of the various forms investigated.

ACKNOWLEDGMENTS

This study was initiated in the laboratory of Mr. William Procter of Bar Harbor, Me., during the summer of 1929 and was completed at the University of Pennsylvania. The writer wishes to express his gratitude to all those who have made this study possible and especially to Dr. D. H. Wenrich for his ever ready counsel and helpful criticism.

TABLE I

Number of species of ciliates comprising infestation of various species of sea urchins examined

Echinoids: Host	Locality	Date of Examination	Nature of Infestation
Strongylocentrotus drōbachiensis (O. F. Müller)	Gulf of St. Lawrence	Sept. 1930	5 species (+2?)
	Roque Is., Me.	Aug. 1930	5 species
	Mt. Desert Is., Me.	July-Aug., 1929–'31	5 species
	Portland, Me.	Nov.-Dec., 1933	4 species
Strongylocentrotus lividus Brd.	Bay of Naples	Aug. 1932	3 species
Echinus esculentus L.	Aberdeen, Scotland	June 1932	———
	Plymouth, England	June 1932	———
Toxopneustes variegatus (Lamarck)	Beaufort, N.C.	April 1931	4 species

The writer wishes also to express his gratitude to the gentlemen directing the laboratories at Naples, Plymouth, Aberdeen, Beaufort, and Woods Hole for their kindness and the abundance of material and equipment supplied him during the course of this investigation.

CILIATES ASSOCIATED WITH SEA URCHINS

In the following notes, the writer has included all the species encountered, for even in cases where the species has previously been described, the present diagnosis includes points in its description which are here noted for the first time.

In describing the flattened ciliates, the ventral surface is taken to be

that one which is most often found in contact with the substratum. When the cytostome is found neither on the ventral nor the dorsal surfaces, but rather on the lateral margin, that margin (edge) is referred to as the oral margin and the opposite side as the aboral margin. This term (oral margin) is also used to designate the side nearest the cytostome. The anterior field is that frontal area, devoid of cilia, which is found on, or bordering, the antero-ventral aboral margin.

Description of Species

Genus CRYPTOCHILIDIUM Schouteden 1906

Kahl (1931) has called attention to the fact that the genus *Cryptochilum* Maupas 1883 is preoccupied by the genus *Cryptochilus* Rafinesque 1815, an hymenopteran; leaving as the next available name, *Cryptochilidium* Schouteden 1906.

Cryptochilidium (*Cryptochilum*) *echini* (Maupas, 1883)

(Plate I. Fig. 3.)

Diagnosis: Average length 83 (45–115)μ, average width 38 (20–55)μ. The body is much flattened, leaf-like with both ends rounded. The region anterior to the middle is exceedingly thin and blade-like, while the region posterior to the middle is somewhat thicker, owing to the food vacuoles which fill this area. The posterior end bears a caudal projection armed with from 4 to 7 bristles, each about 12 to 18 μ in length. Of the two lateral margins, the aboral margin is curved anteriorly while the oral margin is almost straight. The cytostome is located at about the middle of the ventral side of the oral margin. It is particularly conspicuous due to a row of heavy cilia about 12 μ long which are inserted along the length of the right peristomal border. In the buccal cavity, there are found two thin projecting flaps of pellicle which form a pair of non-motile membranes. A small pharynx leads from the buccal chamber to the interior endoplasm. The food vacuoles contain bacteria or fragments of mucosa from sea urchin intestine. A simple contractile vacuole appears posteriorly.

The cytostome is located on the side which appears to be ventral from the fact that the animal uses this surface in contact with the substratum during locomotion. The striations on this ventral surface are parallel with the oral margin and originate in a bare anterior field which runs along the curved aboral margin. The striations on the dorsal surface show a greater curvature than those on the ventral surface because of their origin along the dorsal aboral margin. There are 18 to 20 striations on the ventral as well as on the dorsal surface. The body cilia are about 6 μ long and are not very dense. As the animal rotates

while swimming through its medium, the ventral surface is seen to be somewhat concave with the caudal portion bearing its bristles pointing slightly ventrad.

The spherical macronucleus measures about 20 μ in diameter and is found, in the mid-body region, accompanied by a single micronucleus. A number of individuals were observed in fission or undergoing macronuclear re-organization as described by Daïn (1930).

Host: *Strongylocentrotus lividus* from the Bay of Naples. All the specimens examined contained a rather heavy infestation.

Remarks: The size as observed by the writer is in agreement with that given by Daïn (1930). None of the previous workers who have studied this species seems to have made note of the variable number of posterior bristles or the row of heavy cilia along the right margin of the peristome, although the latter are sometimes indicated in the figures of Daïn. In examining cross-sections of the intestine, there have been found a number of cases where the body of *C. echini* is partially embedded in the intestinal mucosa. This indicates the possibility that *C. echini* is a parasite rather than a commensal in relation with its host. It was discovered that these ciliates will exist for 5 or 6 days in sea water after removal from the sea urchin's intestine, thus suggesting the possibility of oral transmission among sea urchins to account for the widespread prevalence of this infestation.

Cryptochilidium (*Cryptochilum*) *bermudense* (Biggar, 1932)

(Plate I. Fig. 1.)

Diagnosis: Average length 150 (90–185)μ, average width 65 (48–82)μ. The body is broad and greatly flattened anteriorly with food vacuoles and cytostome confined to the posterior region, which tends to be somewhat bulbous and bears a caudal projection armed with bristles from 16 to 25 μ in length. The aboral margin is strongly curved anteriorly while the oral margin is straight and contains the cytostome in a depression posterior to the mid-body region. The cytostomal area is marked by an extensive development of the anterior peristomal margin which bears a row of long cilia about its edge. The cytostome opens into a large vestibule or buccal chamber which has hanging from its roof a field of long cilia. The opening to the pharynx is marked by the presence of flaps of pellicle which form a pair of non-vibratile membranes. A long pharyngeal fiber marks off the region filled with large food vacuoles. The endoplasm about this "food basket" is dense and granular. A simple contractile vacuole, sometimes accompanied by small accessory vacuoles, is found in the region posterior to that containing the food vacuoles; while anteriorly is found a large ovoid ma-

cronucleus, measuring about 40 × 30 μ, accompanied by a single micronucleus. The region anterior to the macronucleus is clear, vacuolated, and very thin.

The physiologically ventral surface is marked by a greater number of striations than the dorsal surface. These striations are parallel with the oral margin and have their origin in part from a short anterior suture, and in part from the anterior field. This frontal area is unique, for projecting from it (and continuous with the anterior field) are numerous lappets which are exceedingly delicate and devoid of cilia.

Conjugation was not observed, although a number of individuals were found demonstrating phases of macronuclear reorganization.

Host: *Toxopneustes variegatus* from Beaufort, N. C. This ciliate was abundant in all specimens of the host examined.

Remarks: *C. bermudiense* found at Beaufort differs from that described from Bermuda by Biggar (1932) in that it is larger and its macronucleus is also of much greater proportions.

Cryptochilidium (*Cryptochilum*) *echinometris* (Biggar, 1932)

(Plate I. Fig. 4.)

Diagnosis: Average length 122 (80–195)μ, average width 46 (33–70)μ. In shape, the body is oblong and much flattened in the anterior region. The oral margin is almost straight while the aboral margin is slightly curved and bears, anteriorly, an anterior field. The posterior region is bulbous and carries a caudal projection armed with bristles. The cytostome is located very near the oral margin on the dorsal surface, slightly posterior to the middle of the body. The pellicle forming the peristomal region is somewhat thickened and devoid of specialized cilia. There is a field of oral cilia which appears to hang from the roof of the buccal cavity.

The ovoid macronucleus is large, measuring about 20 × 30 μ and is found slightly posterior to the mid-body region, accompanied by a single micronucleus. A simple contractile vacuole is located posteriorly.

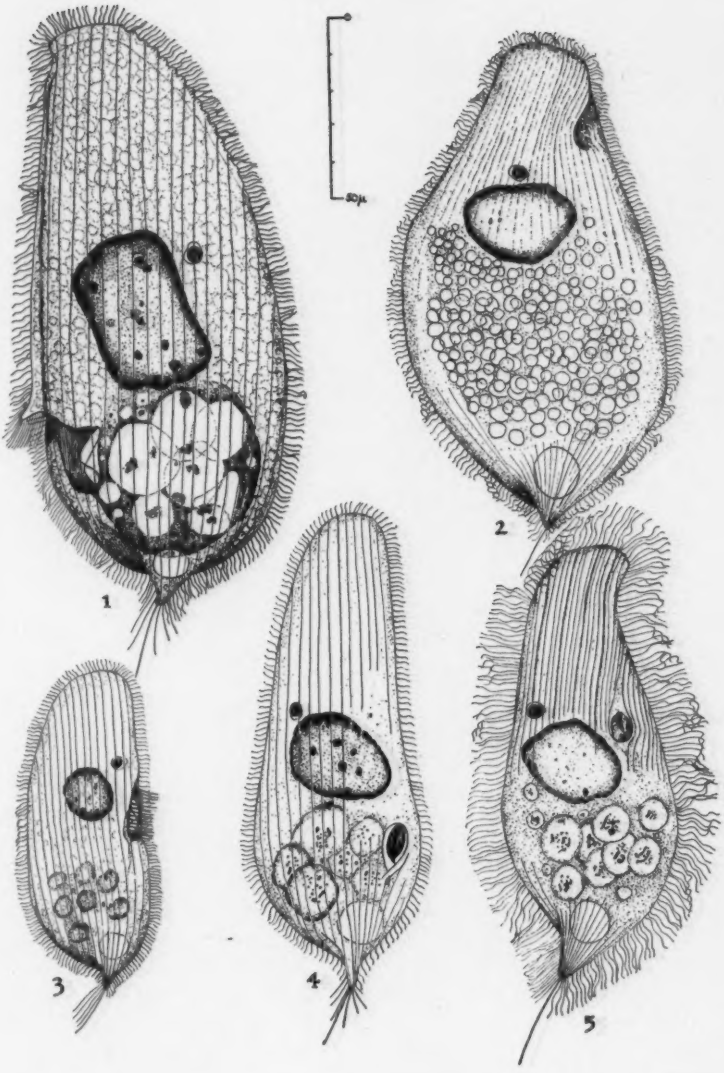
Host: *Toxopneustes variegatus* from Beaufort, N. C. While not very abundant, this species was found in about 50 per cent of the hosts examined.

Remarks: This is undoubtedly the species noted by Bray (1925) under the name of *Cryptochilum echini*.

PLATE I

1. *Cryptochilidium bermudense* (Biggar), as seen from the dorsal surface.
2. *Entodiscus borealis* (Hentschel), dorsal view.
3. *Cryptochilidium echini* (Maupas), ventral view.
4. *Cryptochilidium echinometris* (Biggar), dorsal view.
5. *Cryptochilidium gracile* n. sp., dorsal view.

PLATE I



Cryptochilidium gracile n. sp.

(Plate I. Fig. 5.)

Diagnosis: Average length 100 (78–125)μ, average width 45 (40–55)μ. In shape, the body is roughly oblong with the region anterior to the middle of the body greatly flattened, while that part posterior to the mid-body region is bulbous and bears a caudal projection carrying a bristle about 20μ long. The body is covered with fine, densely placed cilia (12 to 14μ long) arranged in about 64 rows on the ventral surface and 33 rows on the dorsal surface. Several longitudinal ridges are sometimes noted on the surface of the anterior region. The cytostome is located slightly anterior to the dorsal mid-body region, near the oral margin. The anterior field is ventrad. The peristome is unmarked by specialized cilia, although there are indications of cilia lining the buccal cavity. The ovoid macronucleus, located near the middle of the body, averages 30 $\times$ 25μ in size and is accompanied by a single micronucleus. The large food vacuoles, which fill the posterior region, contain bacteria and bits of algæ.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. The infestation seems universal, varying in degree from one individual to another.

Genus ENTODISCUS Madsen, 1931

Entodiscus borealis (Hentschel, 1924)

(Plate I. Fig. 2.)

Synonymy: *Cryptochilum boreale* Hentschel, 1924.
Entodiscus borealis Madsen, 1931.

Diagnosis: Average length 143 (105–170)μ, average width 87 (60–115)μ. The thickness varies from 25–50μ. Body is flattened, disc-shaped, narrowing anteriorly, appearing pyriform when seen broadside. The ventral surface is concave while the dorsal surface is slightly convex. The anterior end is thin, narrow, and blunt, with a bald area or anterior field extending over a part of its surface. Posteriorly, the body is broad and rounded, possessing a caudal projection armed with a fine bristle from 15 to 20μ in length. The body cilia are from 6 to 8μ long and are densely arranged in parallel rows running antero-posteriorly. There are about 36 rows of cilia on the ventral surface and about 26 rows on the dorsal surface. The cytostome is located in the anterior region near the right lateral (oral) margin of the dorsal surface. The entrance to the buccal cavity is guarded by a pair of labial membranes which are opened only during feeding. From the roof of the buccal cavity there hang a group of cilia from 15 to 20μ in length.

The cytostome is further marked by the presence of a supporting rod or "stomato-style" which is imbedded in its upper peristomal border. The macronucleus is ovoid, about $28 \times 22\,\mu$, and is accompanied by a single micronucleus. The macronucleus appears to rest upon a "nest" of food vacuoles which crowd into the region just posterior to it. The food vacuoles seem to contain bacteria, or mucosal cells from the intestinal lining. A simple contractile vacuole, often associated with numerous accessory vacuoles, is found posteriorly.

Conjugation was not observed, although a number of individuals showed evidence of nuclear reorganization and a few individuals were found undergoing fission.

Hosts: In *Strongylocentrotus dröbachiensis* from the Bay of Fundy, *Entodiscus borealis* occurs in great abundance, while in *Echinus esculentus* from the North Sea, the infestation is very slight, occurring in only about 5–15 per cent of the animals examined from the northern waters as reported by Hentschel (1924). It is possible that *E. borealis* is normally associated with *S. dröbachiensis* but is able to infest *E. esculentus* when the two species of sea urchins inhabit the same locality.

Remarks: This species has many features in common with the genus *Entorhipidium* Lynch; it lacks, however, a definite frontal lobe, and trichocysts; and its cytostomal structures seem to be much more highly developed.

Entodiscus indomitus Madsen, 1931

(Plate II. Fig. 8.)

Diagnosis: Average length 85 (69–120)μ, average width 21 (14–40)μ. The body is greatly elongated with the area anterior to the mid-body region greatly flattened, blade-like, and extremely flexible. The anterior edge is thin, broad, and transparent, while the posterior region ends in a tip bearing a bristle about $16\,\mu$ long. The cytostome is located in a small lateral depression at a distance of about one-third of the body length from the anterior end. Out of the cytostome hang two groups of long cilia, an upper and a lower, which seem to be inserted in the back wall of the buccal chamber. A small pharynx leads from the floor of the buccal chamber to the interior endoplasm. The posterior region of the body contains, as a rule, few food vacuoles. A simple contractile vacuole is found, often associated with accessory vacuoles, in the extreme posterior region. The macronucleus is median in position and is always accompanied by a single micronucleus. The body cilia are not particularly dense and are arranged in about 21 parallel rows.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. This species occurs in great abundance but because of its thin body it may be overlooked, for seen edgewise, it is almost invisible.

Remarks: This species undoubtedly has as much in common with the genus *Cryptochilidium* as it has with *Entodiscus*. However, until a detailed morphological study of all the related forms has been completed, the writer will leave this species in the genus to which it was first assigned.

Genus COLPIDIUM Stein, 1860

Colpidium echini Russo, 1914

(Plate II. Fig. 6.)

Diagnosis: Average length 55 (37–64)μ, average width 25 (21–28)μ. The body is cylindrical with both anterior and posterior ends rounded. The cilia are arranged in about 24 rows parallel to the longitudinal axis. The cytostome is obscure, being located in the anterior third of the body as a long narrow groove parallel to the ciliary rows. The right peristomal margin appears to bear a row of long cilia. There seems also to be a group of fine cilia about the entrance to the cytopharynx. A single macronucleus, measuring about $16 \times 16\,\mu$ accompanied by a micronucleus is found in the region anterior to the middle, usually opposite the cytostome. The micronucleus is usually anterior to the macronucleus. The food vacuoles measure about $7\,\mu$ in diameter and fill the endoplasm of the posterior region. A single contractile vacuole is found posteriorly. Individuals were observed conjugating and, in a number of instances, specimens were found demonstrating a type of nuclear reorganization in which the macronucleus had separated into 7 or 8 fragments.

Host: *Strongylocentrotus lividus* from the Bay of Naples. Found, though never abundant, in all specimens of host examined.

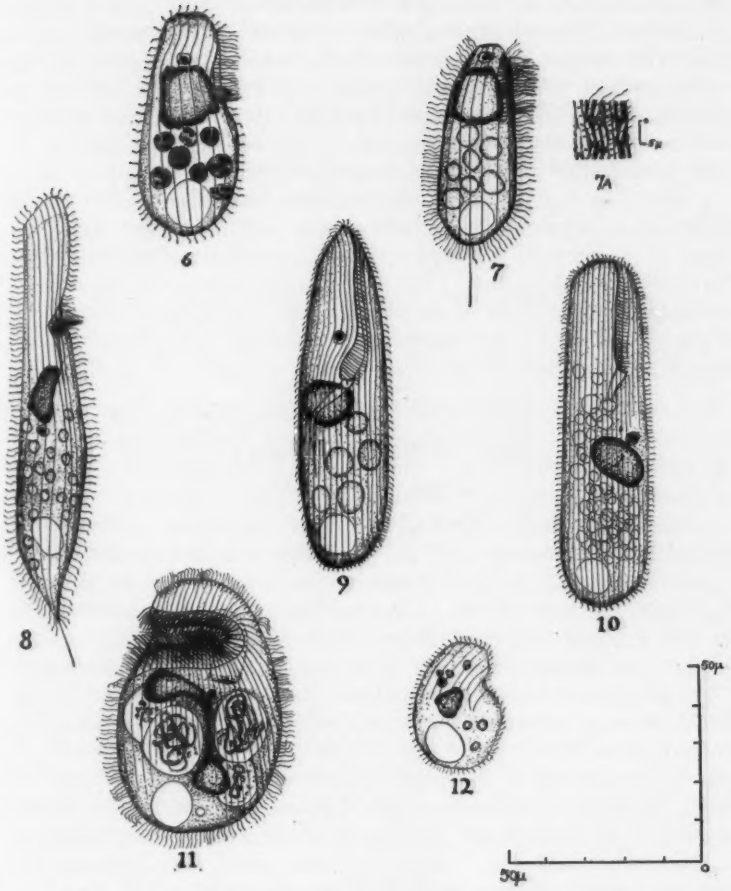
Genus URONEMA Dujardin, 1874

Uronema socialis n. sp.

(Plate II. Fig. 7.)

Diagnosis: Average length 53 (46–70)μ, average width 24 (18–30)μ. The body is cylindrical in shape and of almost constant diameter throughout its length. The anterior field is well marked as a prominent cap on the anterior end devoid of all cilia. The body cilia are about $8\,\mu$ long and are arranged in about 14 to 16 rows which run parallel to the longitudinal axis. Orderly arranged groups of bacteria are found covering the pellicle between the rows of cilia (Plate II, Fig. 7*a*). The posterior end is rounded and bears a terminal bristle 15–20 μ long. The cytostome is located in the anterior third of the body and

PLATE II



6. *Colpidium echini* Russo.
7. *Uronema sociale* n. sp., showing general morphology; *7a.* showing a portion of the pellicle illustrating the appearance of the bacteria which apply themselves to its surface.
8. *Entodiscus indomitus* Madsen.
9. *Anophrys echini* Di Mauro, view showing the cytostomal region.
10. *Anophrys vermiformis* n. sp.
11. *Plagiopyla minuta* n. sp., dorsal view.
12. *Colpoda fragilis* n. sp., dorsal view.

occupies a shallow groove, about 4 μ wide, whose right border is marked by a row of long fine cilia. The cytopharynx is obscure and is located at the base of the oral groove. The ovoid macronucleus measures about $15 \times 16\,\mu$, and is usually located in the anterior region just opposite the cytostome. A single micronucleus is found often in front of the macronucleus. In the middle and posterior regions food vacuoles, about 6 μ in diameter and filled with bacteria (?), are usually abundant. A simple contractile vacuole is located posteriorly.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. This species occurs in great abundance and while it will live and reproduce in sea water, it is always found in greatest abundance in the alimentary tract of its host. The presence of bacteria on the surface, while visible only under oil immersion, is the most helpful criterion for the identification of this species. These bacteria leave the surface of the pellicle upon the death of *U. socialis*.

Genus ANOPHRYS Cohn 1866

Anophrys echini. Di Mauro, 1904

(Plate II. Fig. 9.)

Diagnosis: Length 40 to 65 μ, diameter 12 to 14 μ. The body is cylindrical and elongated with the posterior end rounded while the anterior is somewhat pointed. The body cilia are short and are arranged in longitudinal rows about 2 μ apart. The cytostome is found at the base of a narrow groove which begins along the margin of the anterior region and extends posteriorly about one-fourth of the body length. The right peristomal border is marked by the presence of a row of cilia which increase in length as they approach the cytostome. Leading from the cytostome is a long and well-marked pharynx. The endoplasm in the region posterior to the cytostome is filled with food vacuoles measuring about 7 μ in diameter. The spherical macronucleus measures about 15 μ in diameter and occupies a median position accompanied by a single micronucleus. A simple contractile vacuole is found in the posterior region. No caudal filament was observed.

Host: *Strongylocentrotus lividus* from the Bay of Naples. The writer found these ciliates very rare among the intestinal contents of *S. lividus*.

Anophrys vermiformis n. sp.

(Plate II. Fig. 10.)

Diagnosis: Average length 85 (68–105)μ, average width 27 (20–35)μ. The body is cylindrical with both anterior and posterior ends rounded. The anterior region of the body is particularly motile and

flexible. The cytostome is found in the anterior region at the base of a very shallow and narrow groove. The right peristomal border is marked with a row of cilia which are longer than those of the rest of the body. A short pharynx leads from the base of the oral groove to the interior endoplasm, which is filled posteriorly with food vacuoles. A simple contractile vacuole is found posteriorly. The short body cilia are very fine and are arranged in rows which are about 2 μ apart. The ovoid macronucleus, measuring about 16 $\times$ 10 μ, is located near the middle and is accompanied by a single micronucleus.

Host: *Toxopneustes variegatus* from Beaufort, N. C. Occurrence is fairly common.

Genus PLAGIOPYLA Stein, 1860

Plagiopyla minuta n. sp.

(Plate II. Fig. 11.)

Diagnosis: Average length 65 (50–75)μ, average width 40 (36–46)μ, average thickness 30 μ. In shape this ciliate is bluntly oval as seen from the dorsal or ventral surface. The cytostome is marked by a broad transverse groove which extends two-thirds of the distance across the ventral surface in the region anterior to the middle of the body. The cytopharynx opens from the back of this transverse groove and is lined with very short cilia. About the peristome is a wide striated band bearing stiff, heavy cilia. The food vacuoles are very large and are filled with diatoms and strands of algæ. The macronucleus is large and amorphous, fitting its bulk in the space about the nearby food vacuoles. A single contractile vacuole is found posteriorly. The body cilia are fine, measuring about 6 μ in length, and arranged in rows which are about 1 μ apart in the mid-body region. Short trichocysts are embedded in the ectosarc. The dorsal surface is slightly convex and is marked by a striated band about 3 μ wide, which has its origin as a bare area on the oral margin just anterior to the beginning of the peristomal band of cilia.

Host: *Strongylocentrotus dröbachiensis* from the Bay of Fundy. Occurrence in about 10 per cent of hosts examined, never over 12 individuals found per host.

Genus COLPODA O. F. Müller, 1786

Colpoda fragilis n. sp.

(Plate II. Fig. 12.)

Diagnosis: Average length 33 (23–40)μ, average width 18 (16–23)μ. Body is much flattened and ovoid. The cytostome is located

in a small lateral cleft anterior to the middle. Accompanied by a single micronucleus, the macronucleus measures about 10 μ in diameter and is located slightly anterior to the middle. The body cilia are very short and are arranged in rows about 1.5 μ apart. The endoplasm is usually crowded with food vacuoles and a single contractile vacuole is found posteriorly.

Host: *Toxopneustes variegatus* from Beaufort, N. C. Infestation is abundant when present.

Remarks: Since this species is very sensitive to changes in its environment, observations on the living specimens offered difficulties.

Undetermined Species

There remain two species of ciliates associated with *S. dröbachiensis*, from the Bay of Fundy, whose characteristics could not be determined with sufficient accuracy to permit their identification. One is a member of the genus *Euplotes* and measures about 60 $\times$ 35 μ; the other, a member of the genus *Trichodina*, measures about 50 $\times$ 30 μ. These two ciliates are found in the alimentary canal of *S. dröbachiensis*, on its exterior and also in the nearby seaweed. Never more than 10 to 15 specimens of either species have been found per host.

Discussion

In regard to the taxonomy of these ciliates, there are at least two alternatives: either one may, by examining cross sections and making a critical study of the cytostomal region, find enough evidence to justify the creation of new genera for several of these superficially similar forms (Plate I, Figs. 1–5), or one may, by a study of the gross microscopic anatomy, group these species, within broad limits, under existing genera. This latter course the writer has chosen for the present, for it will be some time before the detailed morphology of all the forms herein described will be sufficiently known to form the basis of a critical judgment.

In considering these forms which are associated with sea urchins, one sees roughly two groups of ciliates involved. The first group consists of the species which the writer has referred to the genera *Cryptochilidium* and *Entodiscus*. These forms are all elongate, with the region anterior to the cytostome exceedingly attenuate and somewhat like a proboscis. The cytostome is either lateral or nearly so and may possess a peristomal margin of specialized cilia. The buccal chamber is often equipped with a pair of membranes and may contain groups of differentiated cilia. The posterior end bears a caudal tip armed with one or more bristles. The body cilia are usually densely arranged in rows

running antero-posteriorly, there being a greater number of striations on the side which is most often in contact with the substratum and hence is termed the physiologically ventral surface. The anterior field is usually found along the anterior margin of this ventral surface. While members of these species will exist for varying lengths of time in sea water of the proper concentration, they nevertheless seem to prefer the environment of the intestinal cæca of their host for active growth and reproduction, and may, therefore, be thought of as being endozoic. The other group consists of the forms referred to the genera *Uronema, Colpidium, Colpoda, Plagiopyla, Euplotes,* and *Trichodina.* These forms are diverse and have many free-living relatives and may be present among the intestinal content of the sea urchin as chance or vagrant ciliates, which, after being engulfed with food, are able to survive in the enteric canal, playing the rôle of scavengers or commensals. One might well argue that this latter group of ciliates, being composed possibly of mere accidental associates, is to be disregarded; however, among all of the possible marine ciliates which might thus be accidentally introduced into the alimentary canal of a sea urchin, it seems significant that these forms described are the only species observed as being involved in this association. Furthermore, it is also of interest to note that of all the members of the Genus *Arbacia* examined, none was found harboring intestinal ciliates, although some species of *Arbacia* are often found in or near localities inhabited by species of *Strongylocentrotus* in which ciliate infestations were found to be universal.

As to the rôle of the first group: they may be considered as commensals with possible pathogenic tendencies. In studying *Cryptochilidium echini* (Maupas) from sectioned material, the writer has observed areas of intestinal mucosa which have been partly penetrated by the ciliates; and from studying *Entodiscus borealis* (Hentschel), there is some slight reason to believe that bits of intestinal mucosa are engulfed by this protozoan, for bodies which closely resemble the nuclei of the cells lining the intestine are often found in the food vacuoles.

The writer now hopes to obtain data on the manner of infestation and of the environmental factors present which allow some of these members of the infestation to be apparently free-living and only occasionally or accidentally associated with their host, while other ciliates of the group seem definitely endo-parasitic. It might also be of interest to discover the factors which bring about such an abundant infestation among the members of the genus *Strongylocentrotus,* while members of the genus *Arbacia* seem devoid of all such ciliate relationships.

SUMMARY

1. Seven species of ciliates are described infesting *Strongylocentrotus dröbachiensis* collected from the Bay of Fundy. This infestation is usually heavy and appears to be universal.

These ciliates are:

Entodiscus borealis (Hentschel)
" *indomitus* Madsen
Cryptochilidium gracile n. sp.
Uronema sociale n. sp.
Plagiopyla minuta n. sp.
Euplotes sp. ?
Trichodina sp. ?

2. Four species of ciliates are described from the intestine of *Toxopneustes variegatus,* collected near Beaufort, N. C.

These ciliates are:

Cryptochilidium bermudense (Biggar)
" *echinometris* (Biggar)
Anophrys vermiformis n. sp.
Colpoda fragilis n. sp.

3. Three species of ciliates are figured from the intestine of *Strongylocentrotus lividus,* taken from the Bay of Naples.

These ciliates are:

Cryptochilidium echini (Maupas)
Anophrys echini Di Mauro
Colpidium echini Russo

4. The relationship of these ciliates with their host is a rather loose one, for the ciliates in question are able to exist for some time in sea water and transmission seems to be orally. The youngest sea urchins examined were lightly infected. There is some evidence to point to a pathogenic rôle on the part of some of the members of the genera *Cryptochilidium* and *Entodiscus.*

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STUDIES ON THE CILIATES FROM SEA URCHINS

II. Entodiscus borealis (Hentschel), (Protozoa, Ciliata), Behavior and Morphology

PHILIP B. A. POWERS

Zoölogical Laboratory, University of Pennsylvania

Introduction

The ciliate *Entodiscus borealis* (Hentschel) is one of five species of ciliates found infesting the alimentary tract of *Strongylocentrotus dröbachiensis* (O. F. Müller) taken from the Bay of Fundy. In an effort to find a key to the taxonomic characteristics of these species, some of which are undoubtedly closely related, this ciliate was selected for intensive study because of its large size and apparent simplicity. Sectioned specimens of *E. borealis* revealed many interesting structural details which are reported in this account of its behavior and morphology.

The material used in this study was collected, in the manner described in the preceding paper, from sea urchins taken off Gaspé, Quebec; Mt. Desert Island, and Portland, Maine.

The writer wishes to acknowledge his indebtedness to Dr. D. H. Wenrich through whose helpful guidance and criticism this study was made possible. The writer is also indebted to W. W. Simonton of the Portland High School, who was able to send live sea urchins to Philadelphia where a complete check was made on all the points herein discussed.

Genus ENTODISCUS Madsen 1931

The generic name *Entodiscus* was selected by Madsen to apply to the two flattened holotrichous ciliates which he found inhabiting the intestine of *Strongylocentrotus dröbachiensis* common to the region about Frederikshavn, Denmark. The larger of these two forms appeared to be identical with a ciliate which had already been described under the name of *Cryptochilum boreale* by Hentschel (1924), who pointed out at the time, that further study might justify the creation of a new genus to receive this species.

The writer has examined some of Mr. Hentschel's preparations and feels that the form described in this paper is the same as that species found by him in *Echinus esculentus* from the Shetland Islands; Millport, and Aberdeen, Scotland.

Entodiscus Borealis (Hentschel)

Observations on Living Material

E. borealis (Fig. 1) is found in great abundance among the liquid contents and food pellets of the sagging loops of the intestinal tract of *Strongylocentrotus dröbachiensis*. Many individuals are found fastened to the walls and in the folds of the intestinal mucosa, while still more are found swimming about in the intestinal fluids which often take on a milky appearance, due to the abundance of ciliates.

E. borealis is very conspicuous due to its large size and the presence of many food vacuoles in its posterior region, which give that region a translucent character. The body shape varies from extremely elongate to oval. In general, the body as seen broadside is pyriform, being greatly flattened as a disc with the dorsal surface slightly arched, while the ventral surface is concave. The anterior region is very thin and narrow, somewhat like a proboscis. The body becomes thicker posteriorly, terminating in a small caudal tip which bears a bristle about 15μ long. The macronucleus consists of a large, homogeneous, egg-shaped mass lying slightly above the middle of the body, seeming to rest in a nest of food vacuoles. The region anterior to the macronucleus is clear and transparent. A large contractile vacuole is found in the posterior region. In one individual whose contractile vacuole was observed during systole, the external vent was seen near the base of the caudal projection. The cytostome is found in a depression near the right anterior margin of the dorsal surface. In living animals, the details of the cytostomal area are obscure; however, in animals which have just died, a number of structures are visible which will be discussed under observations on fixed material.

The body cilia are about 7μ in length, arranged in parallel rows running antero-posteriorly. Because the anterior region of the body is extremely thin and rather narrow, the ciliary rows here are very close together, causing the ciliary movement, which is definitely metachronous, to have the appearance of a series of undulating membranes on the antero-ventral surface. When swimming, these animals rotate on their long axes, moving with steady progress; on the substratum, they move about on their ventral surfaces, often maintaining sufficient suction to defy removal with a pipette. As death ensues, the cilia in the anterior region are the first to cease movement, this cessation of movement proceeding posteriorly until all motion is suspended.

When placed in sea water, these animals will appear normal and will live for various lengths of time, depending upon the temperature and the concentration of the sea water. The writer has kept individuals

of *E. borealis* in hanging drop cultures at 7° C., for periods varying from 15 to 23 days. In these hanging drop cultures, there was no reproductive activity, animals seeming merely to exist. After about the second week in a hanging drop, specimens would begin to show a moribund condition, due largely to failure of the contractile vacuole to empty itself.

Because of the ability of this ciliate to maintain itself for various lengths of time outside the body of its host, and furthermore, because this infestation was found to be universal in all specimens of *S. dröbachiensis* examined, even in young sea urchins from 2 to 4 cm. in diameter; it seems likely that the infestation is contracted, orally, through the feeding habits of the developing echinoid. When the liquid passed out of the anus with the fecal pellets is examined, one often finds a number of *E. borealis*, suggesting that the infestation is perpetuated by the passage of these ciliates from the alimentary tract of one sea urchin to that of another, without any intervening cystic stages.

Of all the living specimens examined, only three ciliates were observed reproducing by fission, although many individuals were observed with their macronucleus separated into two or more parts, suggesting stages in which the macronucleus was undergoing reorganization.

Observations on Fixed Material

Measurements of fifty individuals gave the following dimensions: average length 143 (105–170)μ, average width 87 (60–115)μ; the thickness of the posterior region varied from 25–50μ.

When animals are killed with fumes of iodine or osmic acid, the cilia stand out at right angles to the pellicle and may easily be measured. The cilia vary in length from 7 to 11μ, and are arranged in about 70

EXPLANATIONS OF FIGURES 1 AND 2

1. *Entodiscus borealis* (Hentschel). Dorsal view.

2. Camera lucida drawings from cross-sections of *E. borealis*.

A. View of anterior field showing the crowding together of the ciliary rows on the ventral surface.

B. A section passing through the anterior region. Note the basal granules of the oral cilia (*bas. gra'*).

C. Section through the anterior region of the buccal cavity.

D. Section showing the ventral pellicle of the mid-body region.

E. Showing dorsal pellicle of mid-body region.

F. Portion of pellicle from mid-ventral region. Note delicate longitudinal pellicular fibers running parallel to the rows of basal granules.

ant. fib., anterior fibril of the neuromotor system; *ant. field*, anterior field; *ant. peri.*, anterior peristome; *bas. gra.*, basal granules; *buc. cav.*, buccal cavity; *cil. root.*, ciliary rootlets; *dor. sur.*, dorsal surface; *li. cyt.*, lips of cytostome; *lon. pell.*, longitudinal pellicular thickening; *stom.*, stomatostyle; *ven. sur.*, ventral surface.

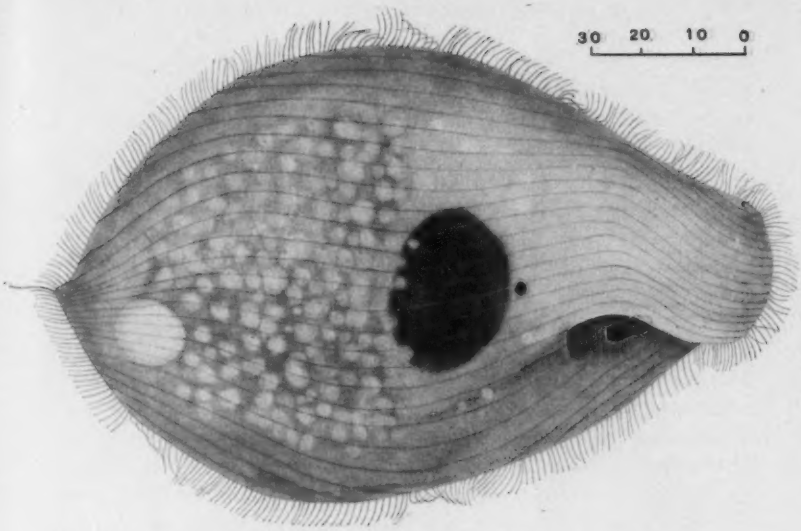

FIG. 1

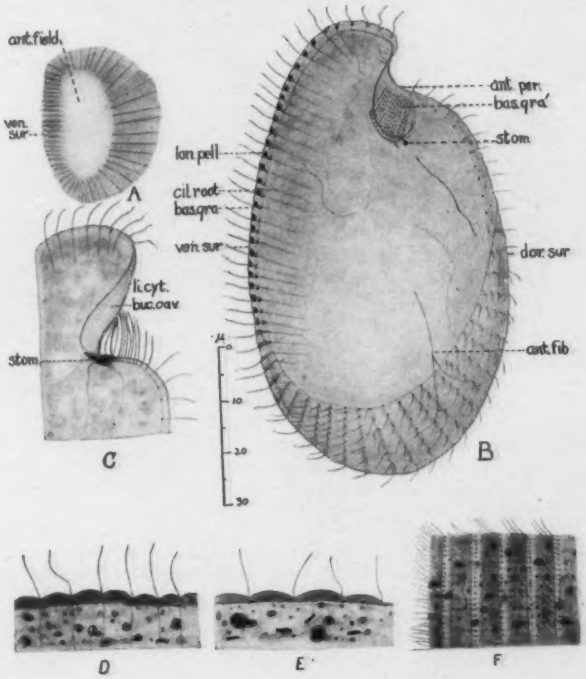

FIG. 2

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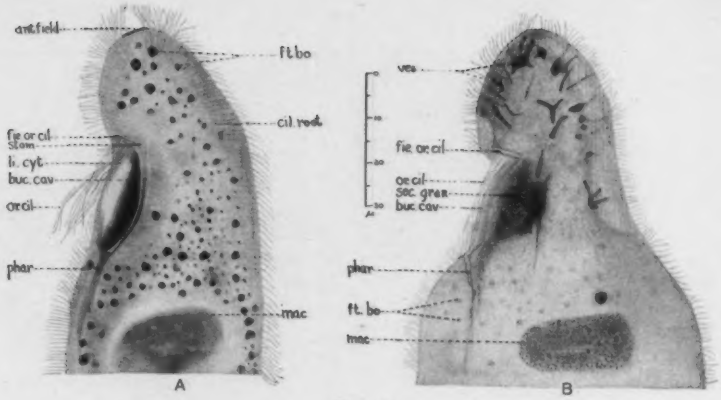
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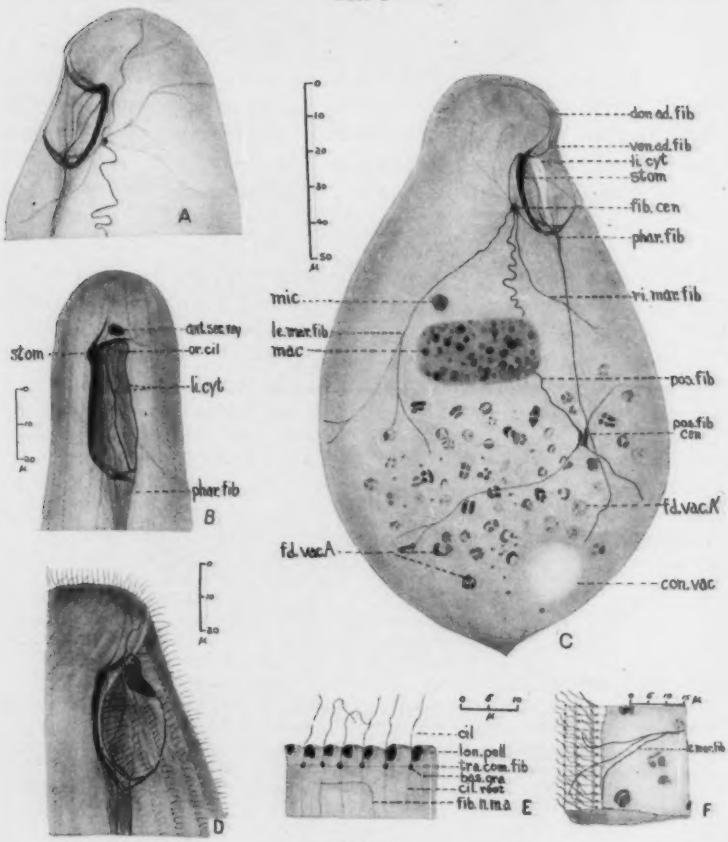
FIG. 3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cil
lon.pell
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bas.gra
cil.root
fib.trans
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FIG. 5

longitudinal rows, each of which lies in a shallow groove, forming a distinct pattern which can readily be seen in the living animals. These rows are much closer together on the ventral surface than they are on the dorsum; there being about 36 rows ventrally and some 24 dorsally. The origin of the ciliary rows is from a bare region on the anterior margin (Fig. 2, *A, ant. field*). This anterior field seems in part comparable to that described for *Entorhipidium echini* by Lynch (1929). In sections of specimens fixed in Champy's, the pellicle of this anterior field is shown to be denser than that of the body surface (Fig. 3, *A*).

The pellicle of the ventral surface is about twice as thick as that of the dorsal surface, and is highly differentiated in its anterior region due to the conspicuous longitudinal pellicular fibrils accompanying each ciliary row (Fig. 2, *B, lon. pell.*). These fibrils, which in the posterior region are very delicate (Fig. 2, *F*), are here very heavy and seem to be either of the nature of a structural "exoskeleton," to protect this region which is most often in contact with the substratum; or they may be of a contractile nature serving as myonemes, explaining in part the mechanism which gives the anterior body region its mobility and allows the creation of the force by which these animals are enabled to hold fast to any smooth surface.

EXPLANATIONS OF FIGURES 3 AND 5

3. Camera lucida drawings of longitudinal sections through cytostomal region showing structures visible after fixation in osmic acid mixtures:

A. Fixed in Champy-Kull. Note the numerous fat droplets.

B. Fixed in Flemming's without acetic (F. W. A.). Note the arrangement of secretory (?) vesicles in the anterior region.

ant. field, anterior field; *buc. cav.,* buccal cavity; *cil. root,* ciliary rootlet; *fie or. cil.,* field of oral cilia; *fa. bo.,* fat bodies or droplets; *li. cyt.,* lips of cytostome; *mac.,* macronucleus; *or. cil.,* oral cilia; *phar.,* pharynx; *sec. gra.,* secretory granules; *stom.,* stomatostyle; *ves.,* secretory vesicles.

5. Details of neuromotor system of *E. borealis.*

A. Cytostomal area as seen through the ventral surface.

B. Camera lucida drawing of cytostome viewed *en face* showing lips fully separated.

C. A complete reconstruction of the neuromotor system as viewed from the dorsal surface.

D. View showing cytostome only partly open.

E. and *F.* Portions of the ventral ectoplasm showing the method by which the body cilia are associated with the fibrils of the neuromotor system.

ant. sec. reg., anterior secretory region; *bas. gra.,* basal granules; *cil.,* cilia; *cil. root.,* ciliary rootlet; *con. vac.,* contractile vacuole; *dor. ad. fib.,* dorsal adoral fiber; *fd. vac. A* and *A',* food vacuoles; *fib. cen.,* fibrillar center; *fib. n. m. a.,* fibril of the neuromotor apparatus; *le. mar. fib.,* left marginal fiber; *li. cyt.,* lips of cytostome; *mac.,* macronucleus; *mic.,* micronucleus; *phar. fib.,* pharyngeal fiber; *pos. fib.,* posterior fiber; *pos. fib. cen.,* posterior fibrillar center; *ri. mar. fib.,* right marginal fiber; *stom.,* stomatostyle; *tra. com. fib.,* transverse commissural fibril.

Just beneath the pellicle along the ciliary rows are the basal granules. These are integrated by means of delicate transverse fibrils (Fig. 5, *E* and *F, tra. com. fib.*), forming a network which is interpreted as a part of the neuromotor system to be discussed later.

The cilia of the ventral surface, particularly of the anterior region, have long ciliary rootlets which penetrate into the endoplasm for about 6–12μ (Fig. 2, *B, cil. root.*). These are very dense in the anterior region and often stain deeply.

No trichocysts were found in the whole mounts or in the sectioned material.

The anterior region is thin, somewhat like a blunt proboscis, with a C-shaped cytostomal groove formed by the downward curve of the right dorsal margin as a slight frontal lobe; making the cytostome partly on the dorsal surface rather than the edge (Figs. 1 and 5, *B* and *D*).

Viewed *en face,* the cytostome, as it appears in the sectioned material, lies in a depression formed by the overhanging frontal lobe. The peristome is formed, in part, by the right margin of this frontal lobe, whose inner surface is the right wall of the buccal cavity. The cilia about this peristomal ridge are from 6–12μ in length and are not much heavier than the body cilia (Fig. 5, *D*). The buccal cavity is guarded by two membranous lips (Fig. 5, *B* and *C, li. cyt.*), which open by separating longitudinally. These lips seem to open along their entire length only during such times as the animal is feeding, being closed or partly so the rest of the time. The lips of the cytostome appear to be formed by a continuation of the delicate membrane lining the buccal cavity. There is found in the roof of the oral depression, just back of the lips of the cytostome where the depression is greatest, an area or field composed of long cilia (Fig. 3, *or. cil.*). These oral cilia measure from 15 to 20μ in length, extending the entire length of the cytostomal region. Judging from the number of basal granules found in this region (Fig. 2, *B, bas. gra'*), some 75 to 100 of these long cilia are inserted in the thickened portion of the membrane (Fig. 3, *fie. or cil.*) which lines the roof of the buccal cavity.

Embedded in the border of the right peristome is a curved supporting rod whose anterior end is forked, and whose slender posterior end terminates among a group of rods which encircle the anterior portion of the pharynx (Fig. 5, *stom.*). This rod appears to form a support for the right wall of the buccal cavity and will be discussed later as a part of the fibrillar system. Because of its obvious nature as a structural element associated with the cytostome, it has been designated the *stomatostyle.*

The pharynx appears to be a definite structure passing downward from the floor of the buccal cavity to disappear near the posterior border of the macronucleus (Fig. 3, *phar.*).

The cytoplasm surrounding the buccal cavity shows two areas which at times are found to be filled with granules suggesting regions associated with the secretion of digestive enzymes. The anterior secretory region (Fig. 5, *B, ant. sec. reg.*) is found just above the thickened pellicle which bears the field of oral cilia. This structure has always been found well defined, as though inclosed by a thin membrane. The larger region of secretory granules is found concentrated near the fibrillar center extending downward along the pharynx (Fig. 3, *B, sec. gran.*).

In the endoplasm are found a number of interesting structures whose demonstration depends largely upon the nature of the fixative used. With the use of Schaudinn's or Bouin's fixative the neuromotor apparatus, nuclear structures, and food vacuoles filling the posterior region are preserved. With the use of osmic acid in the various modifications of F. W. A. (Flemming's without acetic acid) or Champy-Kull technique, there are demonstrated an entirely different set of structures consisting of the secretory regions, fat droplets, and other structures of a lipoidal nature.

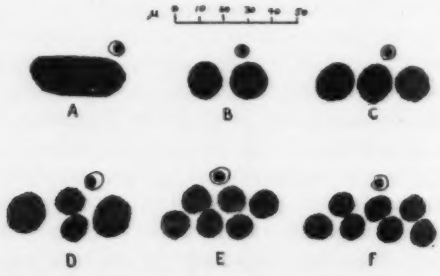
The food vacuoles which usually fill the posterior region of the body, and which are clearly visible in the living organism, appear in sectioned material (fixed in Bouin's) to be of two types: type *A*, those which stain vigorously like nuclear material, and represent newly formed food vacuoles in which the process of assimilation has just begun; and type *A'*, vacuoles which stain faintly, usually with a basic dye, and represent the old food vacuoles whose contents have been assimilated (Fig. 5, *C, fd. vac. A* and *A'*). The food vacuoles of dividing individuals are small and homogeneous, showing that their contents have been assimilated.

The contents of the food vacuoles are rather difficult to identify. In some cases they appear filled with rod-like bodies which resemble bacteria; and in other vacuoles one can see objects which look very much like the nuclei from the intestinal mucosa cells of the host. No algæ or diatoms have been observed in any of the food vacuoles.

Other than food vacuoles, the endoplasm contains large quantities of fat stored as droplets. These are beautifully demonstrated with the use of Sudan III. The fat droplets are found most abundant in the region anterior to the food vacuoles and surrounding the macronucleus (Fig. 3, *A, ft. bo.*). Osmic acid is of value in the demonstration of these fat droplets; however, it preserves all of the other structures of a lipoidal nature and is, therefore, not so specific as Sudan III.

Another structural feature of specimens fixed in F. W. A. is the appearance in the anterior region of a series of vesicular structures (Fig. 3, *B, ves.*) which appear to empty, by means of slender ducts through the pellicle, to the exterior. These vesicles vary in size depending upon their distention. Two or more are often found united to the same duct. They appear to be associated more with the ventral than the dorsal surface. Their contents are of a homogeneous nature and from their reaction with osmic acid suggest a dilute lipoidal secretion. Although traces of them have been found in sections of individuals treated with fixatives other than F. W. A.; all attempts at their demonstration in the living ciliate have been unsuccessful.

The macronucleus is large, measuring on the average about $28 \times 22\mu$ and varying from ovoid to ellipsoid in shape with its long axis at right angles to the longitudinal axis of the animal. In sectioned material, the macronucleus shows a number of deeply-staining spherical bodies of various sizes embedded in its less dense matrix (Fig. 5, *C, mac.*). These deeply staining bodies fail in some cases to stain after the Feulgen reaction, in which case the matrix is lightly colored and contains areas showing the places occupied by the bodies which are deeply stained when treated with iron-hæmatoxylin.



4. Macronucleus of *E. borealis* in various stages of reorganization. *A* to *F*, macronucleus in one to seven parts respectively.

The micronucleus is always single, vesiculate (Fig. 5, *C, mic.*) and usually spherical, measuring about 5μ in diameter; and has always been observed to lie above the macronucleus, never imbedded in it.

A study of about 600 specimens fixed during the day gave but three individuals showing any signs of fission. Division was found to take place as described by Hentschel (1924). A number of individuals exhibited stages in a process of macronuclear reorganization in which the original macronucleus had divided into as many as seven parts, all

arranged in an orderly fashion (Fig. 4, *A* to *F*). In all cases the micronucleus appeared in a normal resting stage. In some 5700 individuals examined, 5538 contained a normal resting macronucleus (Fig. 4, *A*); 103 contained 2 macronuclei (Fig. 4, *B*); 34 contained 3 macronuclei (Fig. 4, *C*); 21 contained 4 macronuclei (Fig. 4, *D*); 1 contained 6 macronuclei (Fig. 4, *E*); and 3 showed 7 macronuclei (Fig. 4, *F*). In every instance, the volume of the macronuclei in each individual, irrespective of number, seems to approximate that of a resting macronucleus.

Fibrillar System

Throughout sections of material fixed in Schaudinn's or Bouin's, there are found traces of a delicate and persistent system of intercommunicating fibers and fibrils. In reconstructing this system as a whole and in its present description, it has been considered as a neuromotor system. This system in *E. boreale* consists of three distinct groups of fibers and associated structures: (1) the stomatostyle with its dorsal and ventral anterior horns and their adoral fibers, the labial fibers, the pharyngeal fiber, and the circumpharyngeal rods; (2) the fibrillar center or motorium with its anterior, posterior, and marginal strands; and the posterior fibrillar center with its associated strands; and (3) the transverse commissural network integrating the cilia.

(1) Within the dorsal margin of the peristome, there is imbedded a curved bar, forked at its anterior end, for which the writer proposes the name *stomatostyle* (Fig. 5, *C, stom.*), because of its obvious nature as a support for the dorsal wall of the buccal cavity. The fork at the anterior end is composed of a dorsal horn which sends a strand of adoral fibers about the antero-dextral border and a ventral horn with fibers running to the basal granules of the oral cilia (Fig. 2, *B, bas. gra'*) as well as to the antero-ventral region. A branch also comes from the base of the ventral horn to become the main fiber of the pharyngeal area (Fig. 5, *C, phar. fib.*). From the manner in which the stomatostyle fragments when injured during sectioning, it appears to consist of a viscous semi-solid enclosed by a membrane. From its position and structure it seems to have a definite function as a supporting bar, or rod, for the frontal lobe which overhangs the buccal cavity. Its posterior end terminates near the pharynx (Fig. 5, *B*). Circumscribing the anterior end of the pharynx, there is a set of at least three heavy fibers or rods which are associated with both the labial membranes of the cytostome, and the posterior region of the stomatostyle (Fig. 5, *B* and *D*). Besides the principal pharyngeal fiber which extends posteriorly to lose itself in the region about the contractile vacuole, there are also

a number of accessory fibrils found in the anterior region of the pharynx (Fig. 5, *B, phar. fib.*). About the lips of the cytostome are found fibers which appear to have a connection with the ventral horn as well as the circumpharyngeal fibers.

Ventrad to the midregion of the stomatostyle is found (2) a fibrillar center or motorium (Fig. 5, *C, fib. cen.*); a clump of darkly staining granules which is a common junction for fibers and fibrils from all parts of the organism. From this center, delicate fibers pass in abundance to the anterior region, which, with its ciliary rootlets, presents a very complex appearance. Likewise, fibers are sent to the stomatostyle, as well as to the walls of the buccal cavity. Clearly defined strands of fibers run to both the right and left margins (Fig. 5 *C, ri.* and *le. mar. fib.*) as well as a large loose strand which passes posteriorly to the posterior fibrillar center (Fig. 5, *C, pos. fib. cen.*). The posterior fibrillar center in turn acts as a distributing point for fibers leading to all parts of the posterior region. The appearance of this structure (*pos. fib. cen.*) varies from individual to individual. In some specimens it is similar to that which has been figured; and in other cases it appears to be composed of a knot of interlocking fibers. The only reason, other than its morphological aspect, to suppose it to be a part of the neuromotor system is suggested by the behavior of this animal when in contact with unfavorable conditions. It has been repeatedly observed that whenever a specimen of *E. borealis* swims head-on into a toxic solution the anterior body region appears to be paralyzed and while in this condition the animal will back away from the unfavorable medium, wheel about, and move to a more suitable environment, solely through the use of the cilia of the posterior body region, suggesting that the posterior fibrillar center may be an auxiliary motor control center for the posterior body region.

The distal ends of most of the fibers described above terminate in fibrils which are closely associated with (3) the transverse commissural fibers found at the base of the ectoplasm and form a network interconnecting all of the basal granules (Fig. 5, *E* and *F*).

The fibers in general show a high degree of contractility as well as elasticity. One often finds in a single fiber regions of irregular diameter, suggesting a partial contraction of the fiber. There is no evidence at present to credit these fibers with functions as myonemes. All observations seem to point to their great elasticity as a device to accommodate the movement of a very flexible body as it progresses through a medium filled with the debris common to a sea urchin's intestinal tract. Thus, the posterior fiber is usually found in a very loose condition with its fiber greatly looped or loosely coiled as though it were capable of great flexibility to accommodate the body movements of the organism.

The neuromotor system, then, consists of three principal groups of structures: (1) the stomatostyle with its associated fibers connecting all parts of the cytostomal area and doubtless the controlling mechanism of the ingestatory apparatus; (2) the anterior and posterior fibrillar centers with their associated fibers leading to all parts of the cytostome and pellicle, of which, because of its greater complexity, the anterior fibrillar center seems more nearly to approach the nature of a coördinating center or motorium than any other structure in the body of the animal; and (3) the pellicular system of commissural fibers which connect the basal granules and coördinate the ciliary movements.

Discussion

One receives the impression, from a study of its internal morphology, that *E. borealis* is a rather highly specialized ciliate; an impression somewhat incongruous to that gained from observing the living animal. Perhaps the most striking feature of its morphology is the region about the cytostome as well as that structure itself. The external features of the mouth are finely constructed of ectoplasm which appears unspecialized and of such a nature as to presuppose feeding habits requiring food of a soft or liquid nature. That is to say, by structural components alone the nature of the food of this ciliate appears limited. When one examines the food vacuoles which so abundantly fill the posterior body region, one can find in them only material suggesting bacteria or bodies somewhat like the nuclei from the cells of the intestinal mucosa. Knowing that this animal has a habit of fastening itself ventrally to the surface of the intestinal mucosa, and trying further to give some function to the secretory vesicles of the anterior region; one is inclined to the consideration of this ciliate as being definitely parasitic and using its anterior vesicles as an agent in the secretion of cytolytic enzymes, thus enabling it to attack the intestinal mucosa of its host.

Closely associated with the cytostome are a number of fibers and rods, obviously of a supporting nature, *i.e.*, the stomatostyle, the fibers of the labial membranes, and the rods about the anterior pharyngeal region. These structures are usually found in a taut condition and seem to be of a more definitely supporting nature than do the fibers associated with the rest of the endoplasm. These latter are very delicate, orderly, and are usually found in a loosely coiled or attenuated condition suggesting a function as conducting fibers or neuroids. These neuroidal fibers radiate from two interconnecting centers, the more complex being in the anterior region while the simpler is found in the posterior region, above the contractile vacuole. It must be borne in mind

that these interpretations of the writer are based solely on the morphological evidence and remain to be proved through actual experimental studies.

Many of the European workers are of the opinion that the transverse commissural fibers alone are sufficient for the control of the body movements and locomotion among the ciliates (Reichenow, 1927); and that any other fibrillar structures are of a supporting nature. On the other hand, most of the workers in America feel that the internal fibrillar system is an integral structure associated with all neuromotor activities. The only experimental evidence with which the writer is familiar, concerning either view, and confirming the latter, is based upon the observations of Taylor (1920), who was able, by means of micro-dissection methods, to present definite experimental data on the function of the neuromotor apparatus in *Euplotes*. As yet, one (Ten Kate, 1927) does not seem justified in making generalizations to cover all of the observations now on record for the fibrillar systems of ciliates. The diversity of the structures described leads one to the feeling that perhaps more than one system of structures has been described in some cases, and that in others, there may be some important features which, due to the technical difficulties involved, have been overlooked. It would seem that the workers on the Continent have been drawing their conclusions from something quite different from that used as a basis of thinking in America.

The view that the fibrillar system is merely structural is based principally on the observations made upon various members of the genus *Balantidium* (Bezzenberger, 1904; Ten Kate, 1927; and Ray, 1932). This is a notable piece of work for it gives us a rather complete picture of the close relationship among the various species of this genus as portrayed by their fibrillar structures, which in many cases are homologous. The interpretation of the fibers in the anterior region of *Balantidium* as being of a supporting or structural nature seems entirely logical. However, this generalization concerning the fibrillar system of *Balantidium* cannot well be applied to all other ciliates, some of which have been shown to be so vastly different morphologically.

It is not inconceivable that a structure such as the stomatostyle of *E. borealis* may possess all three properties ascribed for various fibrillar elements; besides being supportive, it may also be contractile as well as conductile; and likewise, other fibrillar elements may have more than one function. In considering these structures under discussion, one must keep ever in mind the difficulties involved in making observations on these delicate organelles, for their demonstration is dependent upon carefully controlled technique in both fixation and staining. It would

seem that, provided the technique for micro-dissection could be sufficiently refined, *E. borealis* would offer exceptional material for the study of the fibrillar system. This species is obtainable in great abundance at any time of the year and will live in cool sea water of the proper concentration for several weeks.

Summary

1. *Entodiscus borealis* (Hentschel), a ciliate infesting the alimentary tract of *Strongylocentrotus dröbachiensis*, when studied intensively, reveals a number of interesting manners of behavior as well as a series of complicated structures in connection with the cytostome and the fibrillar system.

2. The buccal cavity, guarded by a pair of membranous lips, has hanging from its roof a group of very long cilia. Embedded in the margin along its right wall is found a supporting rod for which the name *stomatostyle* is proposed.

3. Coördinating this stomatostyle as well as other parts of the body are two intricate sets of fibers arranged about two interconnecting centers: the *anterior fibrillar center*, and the *posterior fibrillar center*. These fibrillar structures, along with the transverse commissural fibers, found just beneath the pellicle, are interpreted as a well-developed neuromotor system.

4. After fixation with F. W. A. numerous vesicles, with outlets through the pellicle, are demonstrated in the anterior region. In considering the contents of the food vacuoles, these secretory vesicles suggest themselves as possible agents in producing cytolytic enzymes through whose aid the ciliate is able to attack and ingest portions of the intestinal mucosa of its host. In various regions about the cytostome and the pharynx there are found groups of secretory (?) granules.

5. *E. borealis* will exist for as long as 3 weeks in hanging drop cultures in sea water. Reproductive activity seems to occur only in the body of its host. The infestation appears to be maintained through early oral infection of young sea urchins.

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